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ORIGINAL ARTICLE

Applicability of photobiomodulation and antimicrobial photodynamic therapy for pain management after endodontic treatment: a randomized clinical trial

ABSTRACT

***Aim**: To evaluate the use of photobiomodulation (PBM), alone or combined with antimicrobial photodynamic therapy (aPDT), for pain management after endodontic treatment.*
***Methodology**: Randomized parallel-group superiority trial. The allocation sequence was generated using an online true random number generator. To ensure blinding, participants were informed about the study and the devices that would be used, but not about group allocation. Ninety mandibular molars diagnosed with symptomatic irreversible pulpitis were selected and randomly divided into three groups (n=30): Group 1, control (CT); Group 2, photobiomodulation (PBM); and Group 3, PBM+aPDT. All canals were instrumented by the Reciproc system in a single visit. The incidence and intensity of pain were evaluated before and 6, 12, 24, and 48 hours after endodontic treatment, using a visual analogue scale (VAS). The results were analysed using the Kruskal-Wallis test followed by Dunn's test.*
***Results**: The combination of PBM and aPDT after conventional endodontic treatment resulted in a significant reduction in pain compared to conventional endodontic treatment alone or followed by PBM alone ($p<0.05$). Preoperative pain was significantly greater than pain at 6, 12, 24, and 48 hours after endodontic treatment ($p<0.05$) in all groups. There were no adverse effects attributable to low-level light therapy in any participants.*
***Conclusion**: Our findings suggest that the combination of photobiomodulation and photodynamic therapy is a promising alternative for this purpose.*

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Received 2024, January 9
Accepted 2024, February 22

KEYWORDS *Antimicrobial photodynamic therapy, endodontic treatment, photobiomodulation, postoperative pain.*

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Peer review under responsibility of Società Italiana di Endodonzia
10.32067/GIE.2024.38.01.07
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Introduction

There are two main factors preventing achievement of endodontic treatment goals: the characteristics of the resident microorganisms and the anatomical root configuration, which hinders access to the target region even with recently available technologies (1). Another key issue is postoperative pain (2-4), known as an endodontic flare-up, one of the most common complications and causes of discomfort after endodontic treatment (1). Intracanal laser irradiation is gaining acceptance in endodontic treatment (5) as an adjunct to conventional instrumentation and irrigation protocols, due to its disinfection ability (6, 7). Moreover, studies have reported that the use of laser therapy may result in decreased postoperative pain (8, 9).
Antimicrobial photodynamic therapy (aPDT) can be used as an adjunct to root canal cleaning (10, 11). aPDT combines a visible light source and a photosensitizing agent which, in the presence of oxygen, generates cytotoxic bioproducts such as singlet oxygen, free radicals, and superoxide anions; these damage the microbial cell wall and membrane proteins, leading to cell death (12). Recent publications have shown that aPDT reduces bacterial load, with promising results (13, 14).
Innovative methods have been proposed to reduce postoperative pain and provide greater comfort to patients undergoing endodontic treatment. Various adjuvant therapies are being studied for this purpose (8). Photobiomodulation (PBM) therapy, performed with low-level laser, provides benefits such as analgesia, modulation of the inflammatory process, and cell and tissue regeneration (15, 16). The analgesic and inflammation-modulating effects of PBM are mediated by modulation of prostaglandin synthesis, histamine release, alteration of the pain threshold, increased synthesis of endogenous endorphins, and inhibition of bradykinin synthesis (17).
Given the limited number of published randomized clinical trials in this promising area of research, our study aimed to evaluate whether PBM and aPDT (experimental groups) improved pain management after endodontic treatment compared to a control group not exposed to low-level light therapy. The outcome of interest was control of postoperative pain after endodontic treatment. The null hypothesis was that the PBM and aPDT combined or PBM alone would not influence postoperative pain after endodontic treatment compared to the standard of care.

Materials and Methods

This was a randomized, parallel-group, controlled superiority trial, with an allocation ratio of 1:1.

Eligibility criteria
The study protocol was approved by the relevant institutional ethics committee (Certificate of Submission for Ethical Appraisal: 29404420.6.0000.5374) and was conducted in accordance with the declaration of Helsinki.
The inclusion criteria were as follows:
- Patients diagnosed with irreversible pulpitis (observed as an exaggerated and "persistent" response to cold stimulus) (18) who were not taking any of the following medications: antibiotics, anti-inflammatory agents, analgesics, or immunosuppressants;
- Multirooted mandibular molars (first or second), with moderate curvature (10° to 20°) at mesial canals;
- Crown fit for rubber dam isolation;
- Fully formed roots and foramina;
- Absence of internal/external apical resorptions, dilacerations, and canal calcifications (radiographically confirmed).
The exclusion criteria were:
- Age <18 years, pregnancy, or breastfeeding;
- Periodontal disease on clinical and radiographic examination (changes in bone structure with mineral loss >3 mm) (19);
- Teeth that could not be treated in a single visit (patients who have temporomandibular disorders and/or who cannot endure long treatment period) (20);
- Teeth with extrusion of filling material, as determined on radiographic examination;
- Patients who took any medication after endodontic treatment.

Participants
In total, 90 patients who visited a private dental clinic were invited to participate. All patients were informed of the purpose of the trial and provided written informed consent for participation. Once endodontic treatment was completed, study participants were randomized by an external investigator into 3 groups (n=30). Demographic and dental data, stratified by study groups, are described in Table 1.

Conventional endodontic treatment procedure
All procedures were carried out by a single experienced endodontist. All patients went through a thorough history and clinical and radiographic examination.After radiographs had been obtained (Micro-Image, Indaiatuba, Brazil), thermal pulp testing was performed (Coltene, Cuyahoga Falls, USA). An inferior alveolar nerve block was performed using lidocaine 2% with epinephrine 1:100,000 (Alphacaine™; DFL, Rio de Janeiro, Brazil). The teeth were isolated with a rubber dam (Madeitex, São José dos Campos, Brazil) and a gingival barrier (FGM, Joinville, Brazil). The operative field was disinfected with 2% chlorhexidine (Riohex, São José do Rio Preto, Brazil). The endodontic access cavity preparation was done using carbide burs (#1013, #1015, or FGHL 1016, depending on the tooth; KG Sorensen, Cotia, Brazil). Cervical preflaring was done with FlexMaster Intro rotary files (VDW-Munich, Germany). The working length was determined with an apical locator (VDW-Munich, Germany) and confirmed by radiographic examination. All root canals were instrumented to 0.5 mm short of the root apex with ~21-25 mm C-PILOT hand files (#8, #10 and #15). Reciprocating R-PILOT files (VDW) were used to create the glide path. The canals were shaped with 21-mm or 25-mm VDW RECIPROC R25 files (VDW-Munich, Germany). Each file was used in only three teeth, as recommended elsewhere (21-24).
After shaping, Easy Clean tips (Easy, Belo

Table 1

Demographic and dental data in the study groups

	Randomization			Samples included	
	Groups assessed				
	CT (Conventional endodontic treatment)	**PBM (Photobiomodulation)**	**PBM+aPDT (Antimicrobial photodynamic therapy)**	**Completed**	**Dropouts**
Baseline characteristics	N	N	N	N	N
Total N	30	30	30	90	0
Gender					
Female	22	17	19		
Male	8	13	11		
Type of tooth					
Mandibular first molar	20	19	17		
Mandibular second molar	10	11	13		

Horizonte, Brazil) were used to clean the canal walls through mechanical agitation of the irrigant solution (2.5% sodium hypochlorite (NaOCl) (Asfer, São Paulo, Brazil), 2 mL per canal). Three cycles of agitation (20s each) were performed per canal, with the solution refreshed at each cycle (25). A VDW GOLD motor (Munich, Germany) was used to impart reciprocating motion. Then, 10 mL of 17% EDTA (Fórmula & Ação, São Paulo, Brazil) was placed in each canal for 1 minute. Capillary tips (Ultradent, South Jordan, USA) were used for aspiration, and RECIPROC paper points (VDW, Munich, Germany) were used. Three paper cones, sized according to the reciprocating instrument, were used for each canal.

For obturation, AH Plus filling cement (Dentsply Ltd., Ballaigues, Switzerland) was used, mixed according to the manufacturer's instructions.

The single-cone technique was used, with warm vertical compaction. A single gutta-percha cone (RECIPROC, VDW, Munich, Germany) consistent with the diameter of the file was used. The cone was cut with a plugger, and the cavity was cleaned with a sterile cotton ball soaked in alcohol. Coronal sealing was achieved with a temporary sealer (Coltene, Cuyahoga Falls, USA), and restorative glass ionomer cement (FGM, Joinville, Brazil).

Group 1 (control) – Conventional endodontic treatment (n=30).

Patients received conventional endodontic treatment, without laser therapy.

Group 2 (PBM) – Photobiomodulation (n=30)

After endodontic treatment, photobiomodulation was performed with low-level laser (Therapy EC, DMC, São Carlos, Brazil) in infrared mode (808 nm) with 100 mW power. The energy settings were 2 J per apex, energy density 20.4 J/cm², and a duration of 20 seconds. The laser output spot area is 0.098 cm². Energy was applied in the buccal and lingual regions of the gingival mucosa, close to the root apex (Figure 1).

Group 3 (PBM+aPDT) – Antimicrobial photodynamic therapy and photobiomodulation (n=30).

After instrumentation of the root canal system, aPDT was performed with methylene blue (Chimiolux 0.005%, DMC, São Carlos, Brazil) as photosensitizer and a

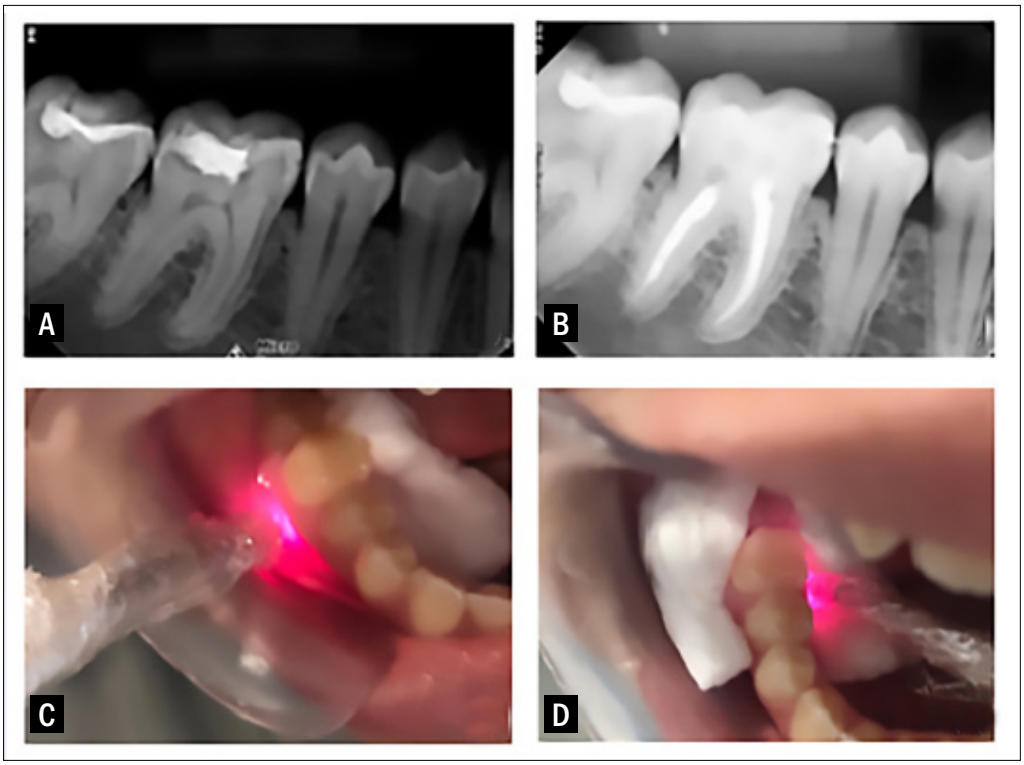


Figure 1
Conventional endodontic treatment combined with photobiomodulation therapy (CT+PBM). **(A)** Initial radiographs; **(B)** after conventional endodontic treatment; **(C)** infrared laser photobiomodulation performed in the buccal region, close to the root apex; **(D)** infrared laser photobiomodulation performed in the lingual region, close to the root apex. Radiographs: Micro-Image, Indaiatuba, Brazil; photographs: Apple iPhone 11 Plus, 2× magnification.

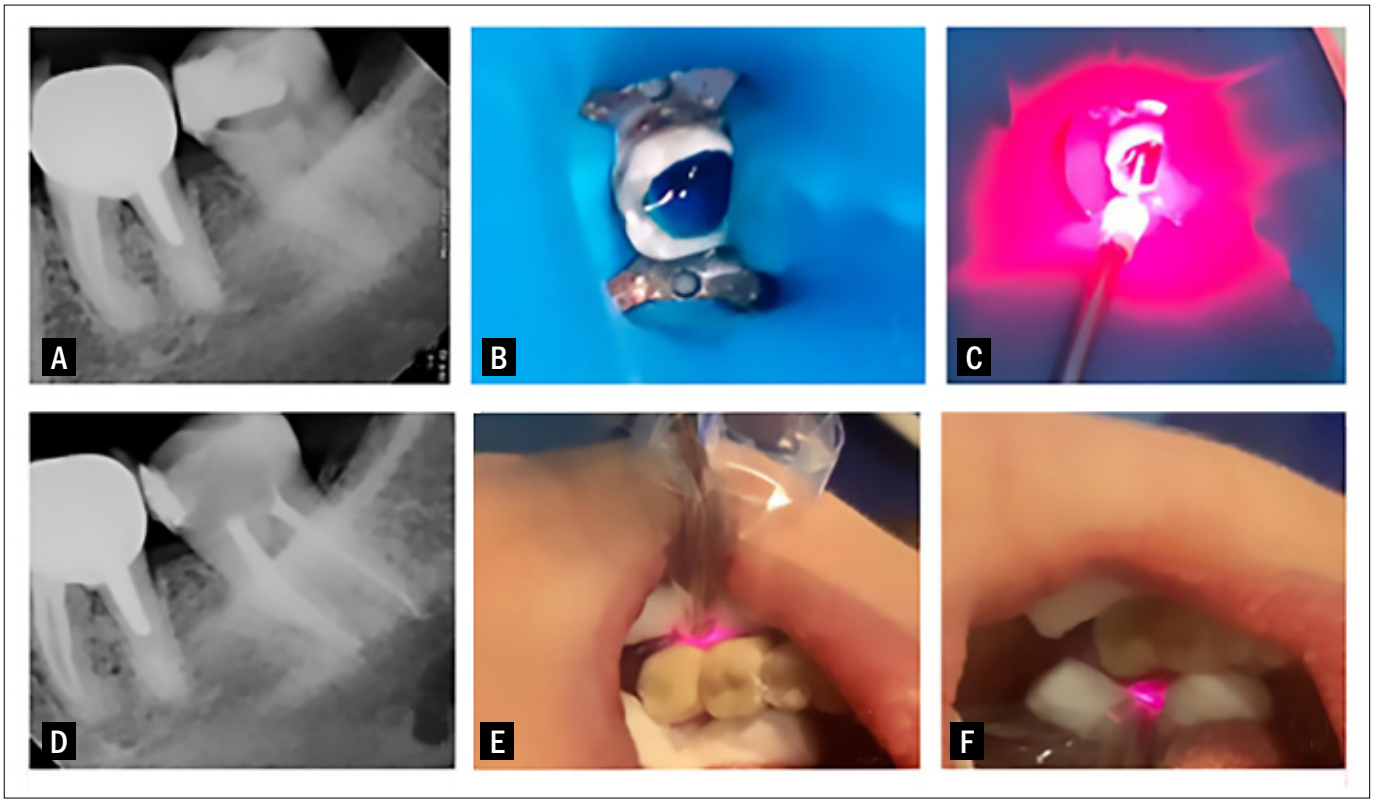


Figure 2
Conventional endodontic treatment combined with antimicrobial photodynamic therapy and photobiomodulation (CT+aPDT+PBM). **(A)** Initial radiographs; **(B)** canal filled with 0.005% methylene blue as photosensitizer; **(C)** antimicrobial photodynamic therapy using a red laser with a coupled fibre optic; **(D)** after endodontic treatment; **(E)** infrared laser photobiomodulation performed in the buccal region, close to the root apex; **(F)** infrared laser photobiomodulation performed in the lingual region, close to the root apex. Radiographs: Micro-Image, Indaiatuba, Brazil; photographs: Apple iPhone 11 Plus, 2× magnification.

pre-irradiation time of 5 minutes. This was followed by irradiation with low-level laser (Therapy EC, DMC, São Carlos, Brazil) in the red (660 nm) wavelength. A fibre optic (DMC, São Carlos, Brazil) was coupled to the laser.
The energy output at the fibre was 5.22 J, with an energy density of 2.899,8 J/cm² and a duration of 90 seconds (Figure 2). Pain assessment was performed at 6 h, 12 h, 24 h, and 48 h after endodontic treatment. Photobiomodulation was performed exactly as in the PBM group. The laser parameters for the PBM and aPDT treatments are described in Table 2.

Outcomes
The outcome of interest was control of postoperative pain after endodontic treatment in the experimental groups (PBM and aPDT).

Sample size
The number of teeth per group was determined through sample size calculation by analysis of variance (ANOVA), with a minimum difference between treatment means=0.10, standard error=0.126, number of treatments=3, statistical power=0.80, and alpha=0.05. The number of teeth per group was thus calculated as 30.

Randomization
A permuted-block randomization strategy with increasing block sizes (2, 4, and 6) was used. Opaque envelopes, each containing the information corresponding to the allocation group, were labelled with sequential numbers. The randomization sequence was generated online (http://www.random.org/).

Blinding
The patients were informed about the study and the devices that would be used, but they were not given information about the group allocation.

Trial registration
The study protocol was registered on the Brazilian Clinical Trials Registry (ReBEC) on January 31, 2022, with access number RBR-7tqy7yw.

Table 2

Irradiation parameters for applications

PBM		aPDT (fibre)	
Wavelength	808 nm	Wavelength	660 nm
Output power	100 mW	Output power	58 mW
Working time	20 s	Working time	90 s
Probe/fibre diameter	600µm	Probe/fibre diameter	1 mm
Spot area	0.098 cm^2	Spot area	0.0018 cm^2
Energy	2 J	Energy	5.22 J
Energy density	20.4 J/cm^2	Energy density	2,899.8 J/cm^2

Statistical analysis

The results were analysed in Bioestat 5.0. The Shapiro-Wilk test was used to verify the assumption of normality. The Kruskal-Wallis test followed by Dunn's test was used for comparisons; significance was set at $p<0.05$.

Results

The selection and randomization of participants are described in Figure 3. Participant recruitment took place from May 1, 2020 through August 1, 2023, and the duration of follow-up was 6 months.

There was a significant reduction in postoperative pain with the use of aPDT+PBM after endodontic treatment was observed compared to control or PBM alone ($p<0.05$) (Table 3). In all groups, preoperative pain was significantly greater than pain at 6, 12, and 24 hours post-treatment ($p<0.05$). After 48 hours, there was no significant difference between groups ($p>0.05$) (Table 3). There were no adverse effects attributable to low-level light therapy in any of the participants.

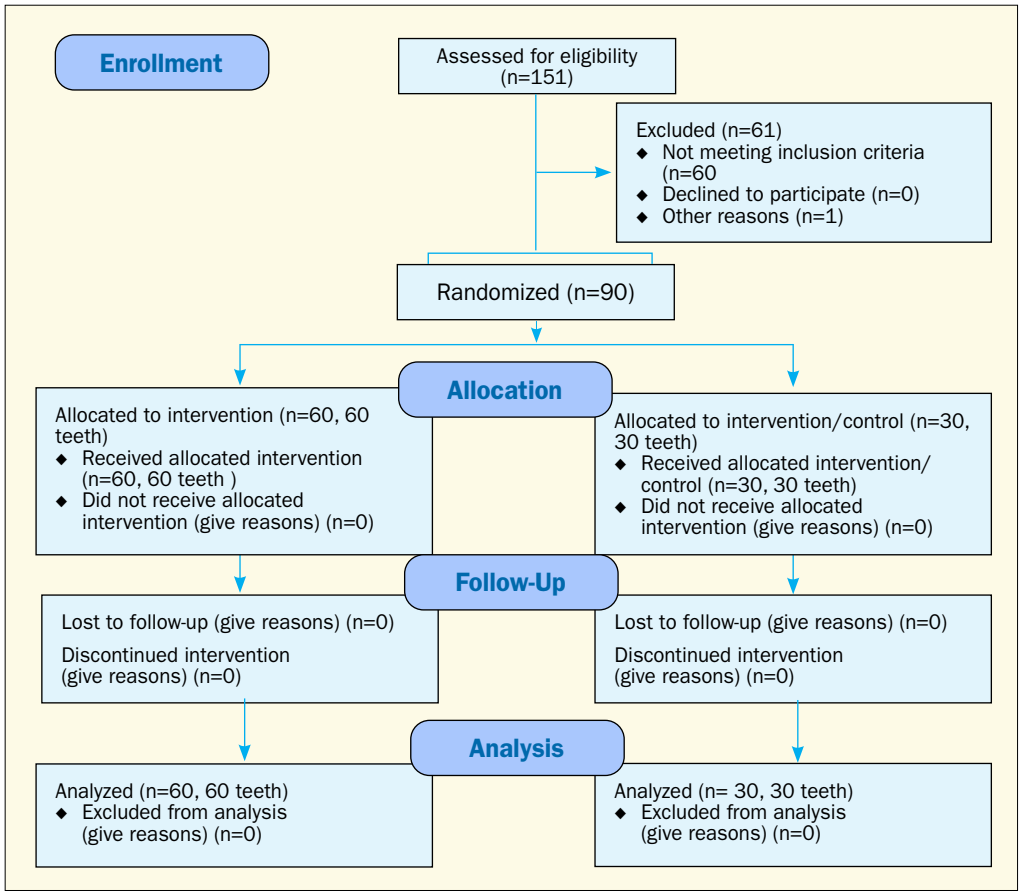
Table 3

Medians, interquartile intervals for VAS scores before and 6, 12, 24, and 48 hours after endodontic treatment in each experimental group (Kruskal-Wallis (Dunn))

	Baseline VAS	VAS 6	VAS 12	VAS 24	VAS 48	(p)
CT	9.00 (3.00)$^{A, a}$	4.00 (4.00)$^{B,1, a}$	3.00 (4.00)$^{B, a}$	2.00 (4.00)$^{B, a}$	0.00 (2.00)$^{B,2, a}$	<0.05
CT + PBM	9.00 (2.00)$^{A, a}$	2.00 (4.00)$^{B,1, ab}$	2.00 (2.00)$^{B,1, ab}$	1.00 (2.00)$^{B, ab}$	0.00 (0.00)$^{B,2, a}$	<0.05
CT + PBM + aPDT	10.00 (2.00)^{A,a}	0.00 (4.00)$^{B, b}$	0.00 (2.00)$^{B, b}$	0.00 (0.25)$^{B, a}$	0.00 (0.00)$^{B, a}$	<0.05
(p)	>0.05	<0.05	<0.05	<0.05	>0.05	

CT: conventional endodontics treatment; CT+PBM: conventional endodontics plus photobiomodulation; CT+PBM + aPDT: conventional endodontics plus photobiomodulation plus antimicrobial photodynamic therapy; VAS: visual analogue scale of pain. VAS 6: VAS score 6 hours after treatment; VAS 12: VAS score 12 hours after treatment; VAS 24: VAS score 24 hours after treatment; VAS 48: VAS score 48 hours after treatment.
Uppercase letters and different numbers in the same row denote statistically significant differences.
Lowercase letters in the same column denote statistically significant differences.

Figure 3
CONSORT flow diagram.



Discussion

Flare-ups remain a challenge for the endodontic practitioner (16) and may have a significant impact on patients' quality of life (25). Therefore, this study was designed to evaluate whether two promising adjunctive modalities, aPDT and PBM could help manage postoperative pain after conventional endodontic treatment. An increased reduction in postoperative pain was observed with the combination of PBM and aPDT after endodontic treatment, suggesting that these adjunctive modalities may have helped manage postoperative pain, thus rejecting the null hypothesis. One possible explanation for the outcome of greater reduction in postoperative pain in the combined aPDT+PBM group is that aPDT would have reduced the microbial load present in the extruded debris, thus enhancing the periapical disinfection achieved by instrumentation of the root canal system. The clinical relevance of these findings lies in the observation that PBM performed immediately after aPDT could modulate inflammation and minimize postoperative pain. In the PBM group without aPDT, inflammation modulation alone was not enough for better postoperative pain control. This is consistent with the findings of Coelho et al. (25), who reported that aPDT decreased postoperative pain at 24h and 72h in single-visit treatment of single-rooted teeth with necrotic pulp.

The literature describes several laser settings for PBM (26). Laser-tissue interactions in the infrared wavelength (810-1.064 nm) depend on the target tissue (1). The PBM protocol of this study used a low-power infrared diode laser (2 J energy), as in previous studies (27). Diode laser has proven to be the most promising approach in terms of postoperative pain reduction. This laser can reach periapical tissues and

modulate inflammation; one possible explanation for its analgesic effect is this deeper tissue penetration and action (28). As noted above, there was a reduction in postoperative pain in the photobiomodulation (PBM) group compared to control, but less so compared to the PBM+aPDT group. aPDT has been used as an adjunct to treatment for disinfection of the root canal system in necrotic teeth, eliminating resistant microorganisms (29). Zargar et al. (30) carried out a study of the microbial flora in cases of irreversible pulpitis and primary endodontic infections. Sixteen microbial species were identified in cases of irreversible pulpitis; a significant burden of microorganisms was identified within the root canals (31), highlighting the importance of antimicrobial techniques such as aPDT. Several parameters of this technique can be modulated (32). The present study was carried out according to the protocol described by Moreira et al. (31), using methylene blue 0.005% as the photosensitizer, with a pre-irradiation dwell time of 5 minutes in each canal. A fibre optic was coupled to the laser unit and placed into the root canal for better light diffusion, reaching the apical third (33). In the present study, the combination of aPDT and PBM resulted in a significant reduction in pain after endodontic treatment. This is consistent with the findings of Vilas-Boas et al. (34), who reported a reduction in postoperative pain after laser therapy in patients with symptomatic apical periodontitis.

Some studies have shown that postoperative pain often occurs during the first 24-48 hours after endodontic treatment (1). To assess pain intensity, we used a simple visual analogue scale (VAS) (35) graded from 0 to 10, where 0 means no pain at all and 10 is the worst pain level imaginable by the patient.

The present trial included patients diagnosed with irreversible pulpitis who had multirooted mandibular molars with moderate curvature. The decision to perform single-visit endodontic treatment aimed to reduce cross-contamination. Furthermore, using a reduced number of instruments with a shorter operative time also causes less instrument fatigue (35). Reciprocating instrumentation has been shown to produce effective results in endodontic treatment (36), with studies reporting satisfactory results regarding postoperative pain (37). Rahbani et al. (38) reported no significant difference in postoperative pain when comparing rotary and reciprocating instrumentation for endodontic therapy.

After preparing the root canal system, some agitation method for irrigation is considered important as a way to improve the removal of debris from inside the canals (39). In the present study, it was decided to use Easy clean in reciprocating mode to agitate the sodium hypochlorite (25). However, even though EDTA at a concentration of 17% is the most commonly used protocol for removing smear layers, it was only applied with conventional irrigation, as its potential for demineralization is known (40).

The main limitation of the study was the 6-month follow-up, was not sufficient to observe e the potential long-term effects of low-level light therapy after endodontic treatment. Another limitation was individual variability in pain thresholds, which hinders sample homogeneity in any study involving pain management.

Further clinical studies should be conducted to assess the utility of aPDT and PBM for pain management after root canal treatment, adding to the evidence base on laser therapies as adjuvants to conventional endodontic treatment. In this study, the combination of aPDT and PBM improved patient comfort by enhancing postoperative pain control.

Conclusion

A combination of aPDT with a 660-nm diode laser and PBM with an 808-nm laser is a promising alternative for pain management after endodontic treatment.

Clinical Relevance

The combination of photobiomodulation and photodynamic therapy is a promising alternative for pain management after endodontic treatment.

Conflict of Interest

The authors have no conflicts of interest.

Acknowledgements/Financial disclosure

The authors have no financial relationships relevant to this article to disclose.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethics approval

This study was approved by the Research Ethics Committees of São Leopoldo Mandic's Faculty (CAAE number: 29404420.6.0000.5374).

Informed consent

All patients were informed of the purpose of the trial and provided written informed consent for participation.

Trial registration

The study protocol was registered on the Brazilian Clinical Trials Registry (ReBEC) on January 31, 2022, with access number RBR-7tqy7yw.

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