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Effect of combined antimicrobial photodynamic therapy and photobiomodulation therapy in the management of recurrent herpes labialis: a randomized controlled trial

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This study aimed to study the effect of combined antimicrobial photodynamic therapy (aPDT) and photobiomodulation therapy (PBMT) in the management of recurrent herpes labialis (RHL). Sixty participants were randomly assigned into three groups. Group 1 (control): 5% Acyclovir was applied as a topical cream, and a non-activating laser was applied. Group 2 (PBMT): PBMT was applied using a low-level laser therapy (LLLT) and a placebo cream. Group 3 (aPDT + PBMT): aPDT using 0.1% methylene blue with PBMT and a placebo cream. A laser diode emitting light at a wavelength of 650 nm and a power output of 100 mW was applied to each spot for 120 s. The parameters of aPDT were a wavelength of 650 nm, with power and energy density parameters set at 100 mW/ 0.1 W and 24 J/cm², respectively. Pain intensity was measured using a visual analog scale (VAS). At the baseline (t_0). After applying the laser (t_1). After 48 h (t_2). After utilizing the laser in the second session (t_3). After 7 days (t_4). The point of healing was the spontaneous shedding of the crust. The aPDT + PBMT group outperforms the control group in reducing pain intensity at t_1 ($p = 0.011$), t_2 ($p = 0.041$), and t_3 ($p = 0.005$). In addition, the aPDT + PBMT group outperformed the PBMT group at t_3 ($p = 0.020$). aPDT + PBMT outperforms control ($p = 0.001$) and PBMT ($p = 0.090$) groups in healing. The findings indicate that aPDT and PBMT offer a promising approach to treating RHL.

Recurrent herpes labialis (RHL) is a common viral infection in the oral and facial areas. RHL is primarily caused by herpes simplex virus type 1 (HSV1)[1]. RHL is a global health problem, occurring in approximately 20 to 40% of young adults[2]. RHL lesions are caused by physical and emotional stress, trauma, ultraviolet light, hormonal changes, and immunosuppression[3]. Prodromal symptoms of the RHL lesions in 46–60% of patients are acute pain, tingling, burning sensation, or itching[1]. RHL lesions progress to multiple small papules, which become fluid-filled vesicles rich in viral particles with a peak viral titer. These vesicles then rupture and eventually ulcerate and crust[1].

HSV-1 lesions are treated with topical application of cream or ointment form of acyclovir, a nucleoside analog antiviral, and the mainstay of treatment[4]. Due to the recurrent nature of HSV infection, prolonged use of antiviral drugs has led to high viral resistance, especially among immunocompromised individuals. Therefore, alternative therapies to antiviral agents should be highlighted[5].

Antimicrobial photodynamic therapy (aPDT) is a modern, non-surgical method for treating lesions affecting the oral and facial areas[6]. aPDT is based on the chemical reaction between a photosensitizer and a light source in the tissue, which causes selective cell damage due to the production of reactive oxygen species[7]. aPDT can be useful in reducing the viral titer in the vesicular phase, and the main advantages appear to include the absence of side effects and drug interactions, which is particularly useful in elderly and immunocompromised patients[8].

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Methylene blue is a phenothiazine photosensitizer that can treat HSV-1 by aPDT with satisfactory results. It has strong light absorption at wavelengths between 630 and 680 nm[9].

Photobiomodulation therapy (PBMT) or low-level laser therapy (LLLT) is based on the analgesic and anti-inflammatory properties of PBMT, and it also enhances biostimulation by inducing fibroblast proliferation, tissue regeneration, immune response, and angiogenesis[10]. The pain-relieving effect is linked to the stimulation of peripheral nerves, which raises ATP levels and helps maintain the stability of cell membranes, resulting in an elevated pain threshold[11]. Furthermore, PBMT is involved in diminishing pain transmission and boosting opioid production. The enhancement of healing is due to improved blood circulation, angiogenesis, and the suppression of prostaglandin E2 (PGE2) production[12]. Various studies have indicated that the wavelengths range from 630 to 980 nm, and the power varies from 20 to 300 mW, with radiation durations spanning 10 s to 15 min for RHL treatment. LLLT is an effective method for preventing RHL without any adverse effects[13]. A combination of aPDT and PBM demonstrated similar advancements in gingival inflammatory and microbiological factors compared to traditional treatment methods. Recently, some advantages of this combined approach have been identified, including the modulation of inflammation, pain alleviation, and a quicker tissue repair process in patients affected by HSV-1[14]. As a result, combining aPDT during the vesicular stage with PBMT during the crusting phase may offer a promising approach for treating RHL lesions. This study aimed to compare aPDT combined with PBMT and PBMT using a laser diode and acyclovir cream for treating RHL lesions by analyzing pain and healing parameters. To the authors' knowledge, it is the first randomized controlled clinical trial that combined aPDT and PBMT. The null hypothesis is that combining aPDT and PBMT in treating RHL lesions would not enhance treatment results.

Materials and methods

Study design and ethical considerations

The study was a double-blinded randomized parallel-group active-controlled trial with three treatment arms. Conducted at the Department of Oral Medicine, Faculty of Dentistry, Damascus University, Syria, it ran from June 2024 to January 2025. Informed consent was secured from participants, ensuring participant confidentiality was maintained. The research adhered rigorously to the CONSORT guidelines[15] and the World Medical Association Declaration of Helsinki concerning human experimentation, as updated in 2013[16]. The Ethics Committee of Damascus University granted approval for the study (N2183), and the trial was registered and authorized in the ISRCTN registry (ISRCTN10142572/https://doi.org/10.1186/ISRCTN10142572) on 20/01/2025.

Sample size calculation

The sample size was determined using G*Power version 3.1.9.4 (Heinrich Hein Universität Düsseldorf, Germany). A total of 60 patients resulted in an effect size f of 0.41, with an 80% power ($1-\beta$ error probability) and a significance level (α error probability) of 0.05. The effect size was derived from a pilot study involving four samples.

Eligibility criteria

Inclusion criteria

1. Healthy participants.
2. Participants older than 18 years.
3. Participants had a history of RHL.
4. Participants had a current active vesicular infection[17].

Exclusion criteria

1. Smokers.
2. Pregnant and breastfeeding women.
3. Diabetic patients.
4. Immunocompromised patients.
5. Participants were allergic to the agents used.
6. Patients had skin lesions that affected the course of healing or interfered with the current study.
7. Patients taking antiviral medications over the four weeks before treatment.
8. Patients taking anti-inflammatory drugs or antibiotics over the two weeks before treatment[17].

Randomization and blinding

The sample was assigned to the three groups randomly by using an envelope filled with 60 cards numbered from 1 to 3. The participants selected a card that indicated the treatment group assigned to them. This study was a double-blinded trial in which the participants and outcome assessors were blinded about group assignments and the study's aim. However, it was not possible to blind the researcher.

Grouping and intervention

The CONSORT flow diagram is illustrated in Fig. 1. Patients referred to the Department of Oral Medicine were evaluated for eligibility. From a total of 73 patients, 60 were randomly assigned into three groups ($n=20$):

- Group 1 (control): 5% Acyclovir was applied as a topical cream (Veramed, Medico Labs., Damascus, Syria), and a non-activating laser was applied.

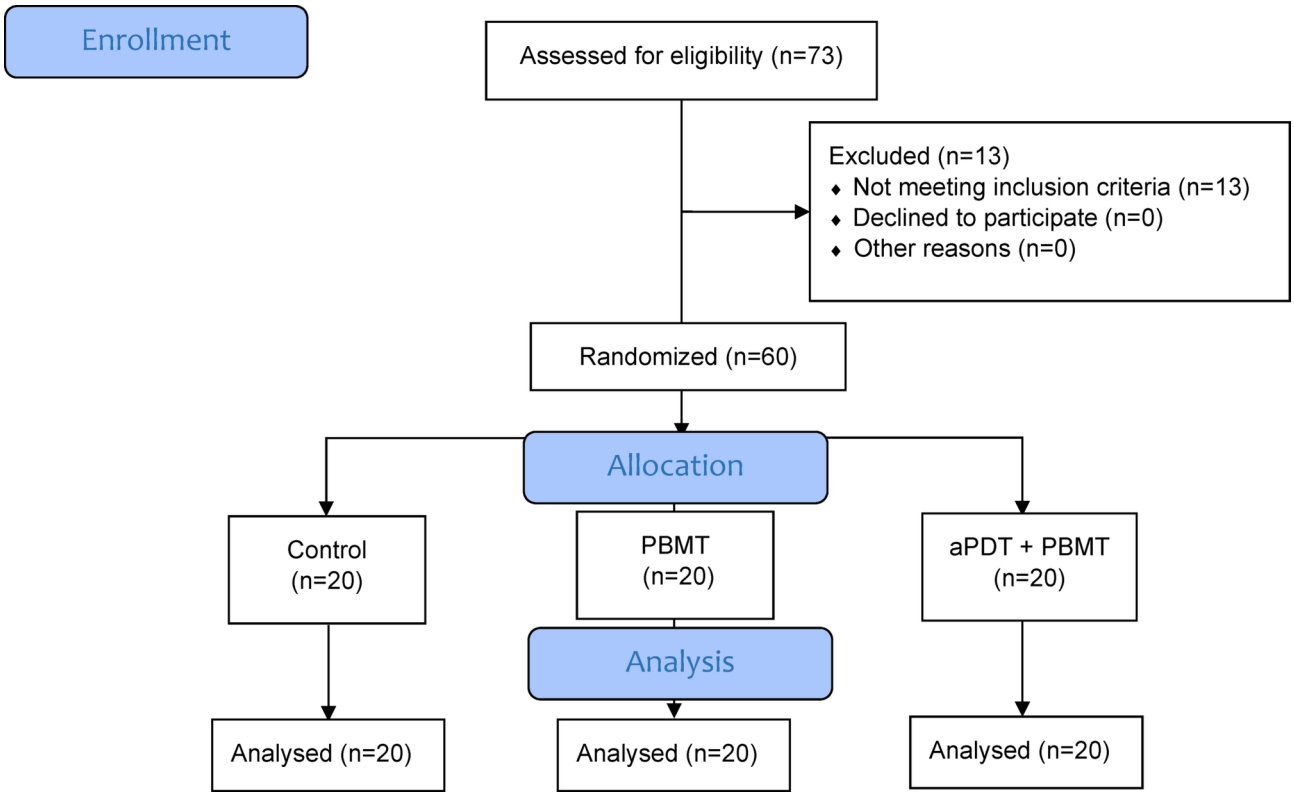

Fig. 1. CONSORT flow diagram.

Wavelength	650 nm
Power	100 mW/ 0.1 W
Beam spot size at target	2.54 cm^2
Power density	40 mW/cm^2
Application mode	Continuous wave
Application technique	Contact
Exposure duration	120 s/spot
Radiant energy	12 J/spot
Radiant exposure (fluence)	4.7 J/cm^2
Number of spots irradiated	Depends on the size of the affected area
Number and frequency of treatment Sessions	Two sessions, 48 hours apart
Total of radiant energy	Depends on the affected area ≥ 12 J per session

Table 1. Settings for LLLT.

- Group 2 (PBMT): PBMT was applied using a LLLT (H1 980 nm Dental Diode Laser, Wuhan Pioon Laser Technology Co.,Ltd,., Hubei, China) and a placebo cream.
- Group 3 (aPDT + PBMT): aPDT using methylene blue with PBMT and a placebo cream.

In the control group, a 5% acyclovir cream was administered to the lesion five times a day over 5 days. A non-activating laser was also used on the lesion to ensure blinding. In the PBMT group, a laser diode emitting light at a wavelength of 650 nm and a power output of 100 mW was applied to each spot for 120 s (Table 1) (Fig. 2). The LLLT was carried out on the first day and again 48 h after the initial application. In addition, the patient received a package including a placebo cream to be applied five times daily[18]. In the aPDT + PBMT group, the area was treated using a cotton ball dipped in sterile saline (SODIUM CHLORIDE 0.9% MIAMED, Miamed Pharmaceutical Industry, Damascus, Syria). A sterile needle tip punctured the vesicle to facilitate the drainage of its contents. The area was dried with sterile gauze within the lesion's borders, avoiding pressure or traction. The herpetic lesion was treated with a 0.01% methylene blue photosensitizer (Citrate Methylene Blue, 0.01% (w/v) 50mL, White labs., California, United States)[9]. After five minutes, the laser therapy was applied (Table 1). Any remaining traces of the photosensitizer were removed with a cotton ball soaked in sterile saline, again

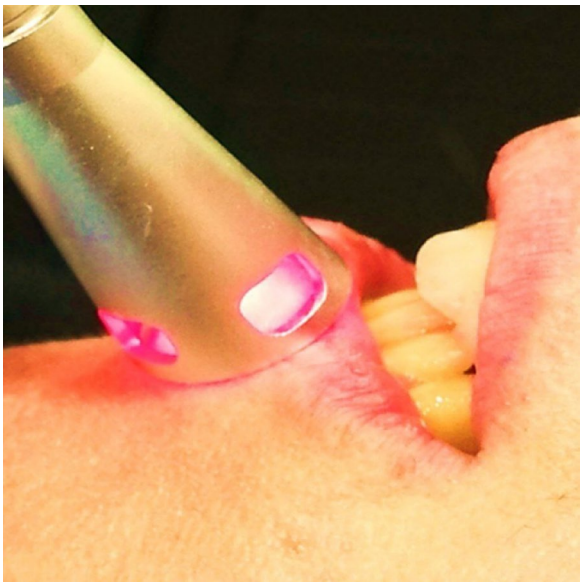

Fig. 2. Applying PBMT utilizing a LLLT.

Photosensitizer	Methylene blue
Concentration	0.01%
Wavelength	650 nm
Power	100 mW/ 0.1 W
Beam spot size at target	0.5 cm^2
Power density	200 mW/cm^2
Application mode	Continuous wave
Application technique	Contact
Exposure duration	120 s/spot
Radiant energy	12 J/spot
Radiant exposure (fluence)	24 J/cm^2
Number of spots irradiated	Depends on the size of the affected area
Number and frequency of treatment Sessions	Single session
Total of radiant energy	Depends on the affected area ≥ 12 J per session

Table 2. Settings for aPDT session.

without pressure or traction. In addition, the LLLT, without the photosensitizer, was utilized 48 h after the aPDT (Table 2), and the patient was provided with a placebo cream for application five times a day[19,20]. The parameters of aPDT were a wavelength of 650 nm, with power and energy density parameters set at 100 mW/ 0.1 W and 24 J/cm^2, respectively[9].

Outcome measures

Outcome measures were recorded by two blinded investigators (ICC > 0.8). The outcome measures were as follows:

Pain intensity

Pain intensity was measured using a visual analog scale (VAS), at the following time points:

- At the baseline (t_0).
- After applying the laser in the first session (t_1).
- Before applying the laser in the second session, after 48 h (t_2).
- After applying the laser in the second session (t_3).
- After 7 days of follow-up (t_4).

VAS scores were as follows:

- 0 = No pain.

Groups	*n*	Male		Female		Age	
		n	%	*N*	%	Mean	SD
Control group	20	1	5.00	19	95.00	27.70	9.35
PBMT	20	5	25.00	15	75.00	25.80	6.56
aPDT + PBMT	20	2	10.00	18	90.00	23.00	4.28
Total	60	8	13.33	52	86.67	25.50	7.19

Table 3. The demographic traits of the participants.

Time points	Groups	Mean ± SD	Minimum	Maximum	*p*-value
t_0	Control	4.20 ± 2.17	1	8	0.838
	PBMT	4.70 ± 2.54	1	8	
	aPDT + PBMT	4.35 ± 2.30	1	10	
t_1	Control	3.85 ± 2.48	0	8	**0.013***
	PBMT	2.45 ± 2.24	0	7	
	aPDT + PBMT	1.6 ± 1.79	0	5	
t_2	Control	1.50 ± 1.57	0	5	**0.038***
	PBMT	0.85 ± 1.66	0	6	
	aPDT + PBMT	0.55 ± 1.32	0	5	
t_3	Control	1.40 ± 1.47	0	4	**0.003***
	PBMT	0.45 ± 1.00	0	3	
	aPDT + PBMT	0.25 ± 0.72	0	3	
t_4	Control	0.00 ± 0.00	0	0	1.000
	PBMT	0.00 ± 0.00	0	0	
	aPDT + PBMT	0.00 ± 0.00	0	0	

Table 4. Descriptive statistics and the results of Kruskal-Wallis test for comparison between mean VAS scores at different time points. Significant values are in bold ($p < 0.05$).

- 1–3 = Mild pain.
- 4–6 = Moderate pain.
- 7–9 = Severe pain.
- 10 = Worst pain possible[21].

Lesion healing

The point of healing was considered to be the spontaneous shedding of the crust from the lesion. Patients were followed up, and the day the crust fell off spontaneously was recorded[22].

Statistical analysis

Statistical analysis was conducted using IBM SPSS software version 24 (IBM SPSS Statistics® version 24, IBM Corp., New York, USA). Descriptive statistics included the frequency, percentage, mean, standard deviation, as well as the minimum and maximum values. The Kolmogorov–Smirnov test was utilized to assess the normality of the data, and a Kruskal-Wallis test was subsequently performed to compare outcome measures at various time points across three groups. The significance level was established at 0.05 ($p < 0.05$).

Results

The demographic traits of the participants in the study are shown in Table 3. Most participants were female ($n = 52$; 86.67%), with an average age of 25.50 years (SD 7.19; age range 19–50 years). Table 4 presents descriptive statistics and the results of the Kruskal-Wallis test for comparison between mean VAS scores at different time points. The mean VAS scores of the study groups were homogenous at the baseline since no statistically significant difference was noted at t0 ($p = 0.838$) (Table 4). The mean vas scores at t_0 were 4.20 ± 2.17, 4.70 ± 2.54, and 4.35 ± 2.30 in the control, PBMT, aPDT + PBMT groups, respectively, which become 0.00 ± 0.00 ($p = 1.000$) (Tables 4 and 5). The aPDT + PBMT group outperforms the control group in reducing pain intensity at t_1 ($p = 0.011$), t_2 ($p = 0.041$), and t_3 ($p = 0.005$) (Table 5). In addition, the aPDT + PBMT group outperformed the PBMT group at t_3 ($p = 0.020$) (Table 5). Descriptive statistics and the results of the Kruskal-Wallis test for comparison between mean days of lesion healing are presented in Table 6. The mean days of lesion healing were 4.75 ± 1.25, 4.05 ± 1.32, and 3.20 ± 1.06 in the control, PBMT, aPDT + PBMT groups, respectively ($p = 0.001$), with aPDT + PBMT outperforms control ($p = 0.001$) and PBMT ($p = 0.090$) groups (Table 7) (Figs. 3 and 4).

Time points	Multiple pairwise comparisons	Test statistic	*p*-value
t_1	Control vs. PBMT	9.525	0.238
	Control vs. aPDT + PBMT	15.825	**0.011***
	PBMT vs. aPDT + PBMT	6.300	0.738
t_2	Control vs. PBMT	8.725	0.201
	Control vs. aPDT + PBMT	11.750	**0.041***
	PBMT vs. aPDT + PBMT	3.025	1.000
t_3	Control vs. PBMT	12.300	**0.020***
	Control vs. aPDT + PBMT	14.250	**0.005***
	PBMT vs. aPDT + PBMT	1.950	1.000

Table 5. Multiple pairwise comparisons of mean VAS scores between groups. Significant values are in bold ($p < 0.05$).

Groups	Mean ± SD	Minimum	Maximum	*p*-value
Control	4.75 ± 1.25	3	8	**0.001***
PBMT	4.05 ± 1.32	2	6	
aPDT + PBMT	3.20 ± 1.06	2	6	

Table 6. Descriptive statistics and the results of Kruskal-Wallis test for comparison between mean days of lesion healing. Significant values are in bold ($p < 0.05$).

Multiple pairwise comparisons	Test statistic	*p*-value
Control vs. PBMT	7.950	0.413
Control vs. aPDT + PBMT	19.575	**0.001***
PBMT vs. aPDT + PBMT	11.625	**0.090***

Table 7. Multiple pairwise comparisons of mean days of lesion healing between groups. Significant values are in bold ($p < 0.05$).

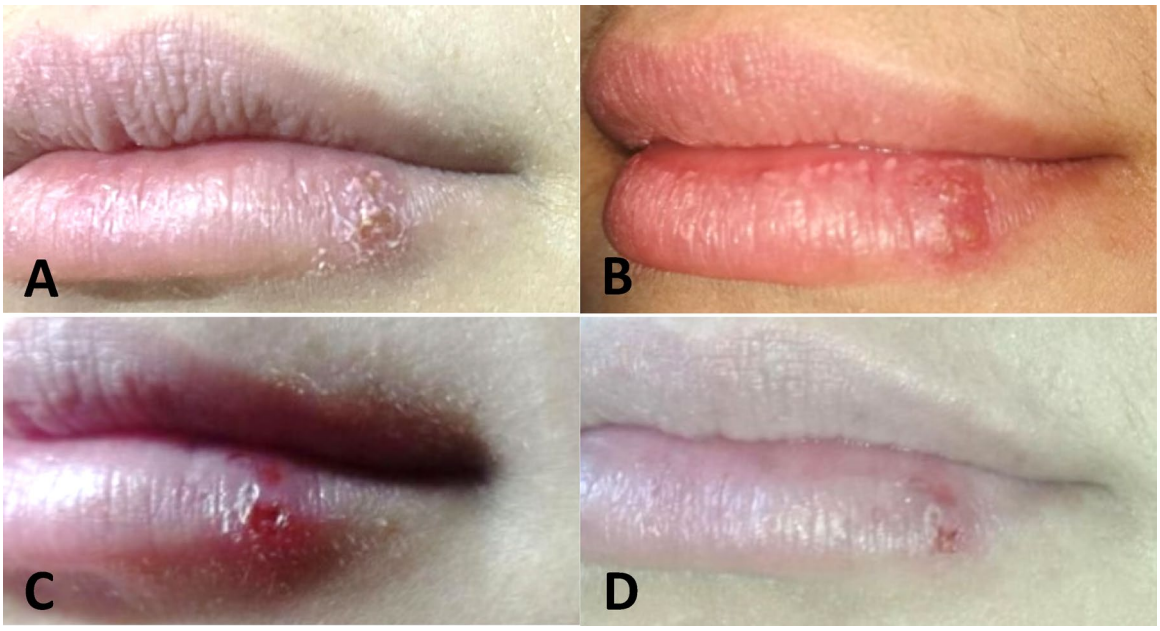

Fig. 3. Healing stages of PBMT group. (**A**) The 1st day. (**B**) The 2nd day. (**C**) The 3rd day. (**D**) The 6th day.

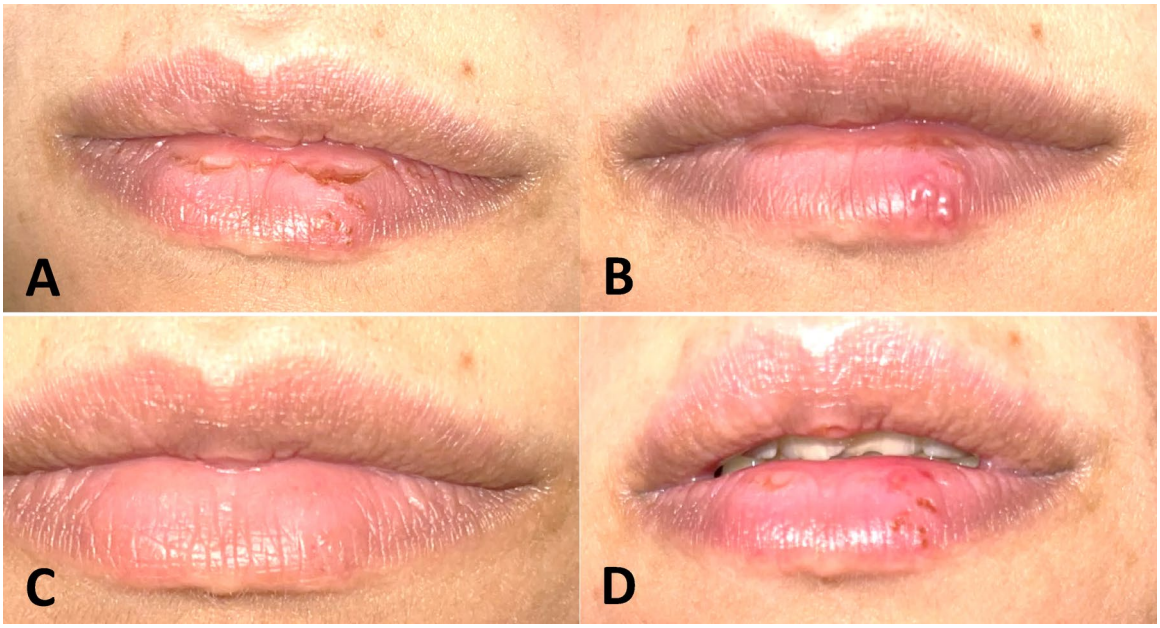

Fig. 4. Healing stages of aPDT + PBMT group. (**A**) The 1st day. (**B**) The 2nd day. (**C**) The 3rd day. (**D**) The 6th day.

Discussion

This study aimed to evaluate the therapeutic efficacy of aPDT combined with PBMT and compare it with PBMT alone and 5% acyclovir cream in treating RHL lesions. The management of RHL lesions is not aimed at eradicating the virus but rather at alleviating clinical symptoms, reducing recurrence, and limiting viral shedding and complications[23]. Therapy was initiated during the vesicular stage of the lesions. This phase was selected because it produces the highest viral load, as the vesicles are filled with fluid that harbors a significant number of viral particles, making it the most noticeable and distressing phase for patients[24]. Patients were given only two laser sessions with a 48-hour interval to achieve therapeutic results with the fewest possible sessions, thus reducing the burden on the patient and the doctor[19,20]. aPDT primarily focuses on producing significant quantities of reactive oxygen species (ROS) that harm bacterial cells by inducing double-stranded DNA breaks, promoting lipid peroxidation, and resulting in protein carbonylation. Nonetheless, the production of ROS can also lead to harm in various cellular organelles and functions, which may eventually disturb normal physiological processes[25,26]. Recently, Hamblin et al. proposed a third photochemical pathway (Type III), whereby light-induced inactivation of bacteria in the absence of oxygen can occur via the generation of photosensitizing anionic radicals or inorganic radicals, enhancing therapeutic efficacy in oxygen-deficient environments[27]. Previous studies of aPDT therapy have successfully used a wide range of photosensitizers. Each provides a different mechanism of viral inactivation, depending on the photosensitizer's affinity for viral components[28,29]. The methylene blue used in our study inactivates viral genetic material, not viral susceptibility, which is of interest in the case of oral herpes because the infection has already occurred[30]. Khalil et al.[31] review suggested that previous studies treating RHL lesions with aPDT used methylene blue due to its activity, low cost, and easy availability. Methylene blue is an FDA-approved dye that absorbs red light at a peak absorption of 660 nm and can inactivate the virus by causing oxidative damage to its DNA. Methylene blue was applied at low concentrations of 0.01% in the current study because it does not cause tissue toxicity or side effects[32,33]. Although there are a variety of light sources used in aPDT, such as tungsten lamps, quartz halogen lamps, xenon lamps, ultraviolet light, and light-emitting diodes, LLLTs are the most common source of photosensitization in viral infections because they emit a single wavelength, which provides a favorable interaction with the sensitizer, in addition to the possibility of accurately calculating the radiation dose[34]. The Arndt–Schultz curve describes the dose-dependent effects of LLLT. It indicates that weak stimulation increases physiological activity, moderate stimulation inhibits it, and intense stimulation eliminates it[10,18]. It emphasizes the importance of an appropriate and calculated dose. Fluences between 1 and 5 J/cm2 are optimal for achieving an optimal biological response[18]. There is no ideal wavelength. The wavelength between 808 –780 nm is effective for the preventive treatment of RHL in the latent phase, while visible red laser light between 700 –600 nm is more effective in the prodromal and cortical phases[35]. Despite promising results on aPDT for HSV-1 infection, there is still no consensus on the type of photosensitizer for the appropriate irradiation parameters to be well established as clinical protocols for treating RHLs[36]. This study selected the value of 24 J/cm² based on existing scientific research regarding the application of methylene blue aPDT for addressing oral lesions, considering factors like the concentration of the photosensitizer, the type of tissue involved, and the nature of the viral infection[37]. A review conducted by Cecatto et al.[38] indicated that the energy density employed in clinical aPDT studies varied from 6 to 18 J/cm², underlining the inconsistency of values due to differing protocols and the absence of a standardized, approved guideline. Conversely, other

research, such as that by Carrera et al.[37], has highlighted the effectiveness of applying higher energy densities of up to 30 J/cm² with methylene blue, especially in oral microbial infections. The findings from a study by Pinheiro et al.[39] support this methodology, showing significant efficacy in inhibiting HSV-1 when utilizing aPDT with methylene blue at a dose of 30 J/cm² while ensuring cell viability in laboratory settings. Therefore, the value of 24 J/cm² was chosen to guarantee adequate ROS production essential for the inactivation of HSV-1 while maintaining the integrity of oral tissue in a safe clinical environment.

In recent years, LLLT has increasingly been proposed as a viable option for pain relief. LLLT pain relief occurs gradually and cumulatively, requiring multiple sessions. Several mechanisms of pain relief have been studied by LLLT, including suppression of substance P and bradykinin activity, increased release of β-endorphin, serotonin, and synaptic activity, as well as improved local microcirculation and tissue oxygenation. It also helps shift metabolism from anaerobic to aerobic pathways and reduces the production of acidic metabolites that stimulate pain receptors[40]. In addition, LLLT stimulates peripheral nerves, which in turn increases adenosine triphosphate (ATP) concentration and maintains cell membrane stability, leading to an elevated pain threshold[41]. Antiviral Mechanism Photokinetic inactivation of viruses has a multi-target mode of action, as ROS can interact with DNA/RNA, proteins, and viral lipids. Furthermore, it may interfere with specific stages of the viral life cycle. Viruses at different stages of their life cycle likely exhibit different sensitivities to this inactivation. The non-targeted mechanism of damage induced by photokinetic therapy is one of its advantages over the photoinactivation of viruses, as this non-targeted mechanism of action is less likely to induce viral resistance[42]. Methylene blue works by disrupting viral DNA; in other words, the binding of methylene blue to red laser light is capable of unraveling viral DNA. Methylene blue is known to bind to DNA in two main ways: either by binding to the outer helix or by intercalation, particularly in guanine- and cytosine-rich regions[22]. This dye is known to undergo both type I and type II photoreactions. Direct electron transfer is likely to occur, leading to DNA strand breakage oxygen or at low oxygen concentrations. In the presence of oxygen, photooxidation occurs through a type II mechanism; this has been demonstrated by the formation of 8-hydroxylamine in nucleic acids upon photo treatment with methylene blue. The damage caused by type I and type II photoreactions is not limited to DNA/RNA; methylene blue also damages viral surface structures, such as proteins[43].

Based on our study's findings, aPDT with 0.01% methylene blue demonstrated greater effectiveness in alleviating the intensity of pain linked to RHL lesions when compared to acyclovir cream, both right after the initial treatment and following the use of LLLT in the second session. Furthermore, it significantly sped up the relief of pain. The findings contrast with those of Ramalho et al.[22], who did not observe a significant difference in pain relief between aPDT and acyclovir cream. Participants were randomly divided into three groups: aPDT (low-power laser, 660 nm, 40 mW, 120 J/cm2, 4.8 J, 120 s per point) with methylene blue (0.005%) used as the photosensitizing agent; 5% Acyclovir; and aPDT combined with 5% Acyclovir. This discrepancy may be attributed to several factors, including the pain scale employed. In our research, we utilized the VAS, known for its precision in assessing pain intensity across a broad spectrum, ranging from 0 to 10. In contrast, Ramalho et al.[22] adopted a different scale with limited scores: 0 = no pain, 1 = mild pain, 2 = moderate pain, and 3 = severe pain. Given that most RHL lesions typically present mild to moderate pain, the limited scoring system used by Ramalho et al.[22] may lack the sensitivity required to detect differences between the treatment groups. Alongside the variation in application protocols, our study involved a combination of aPDT and PBMT. aPDT was administered with methylene blue during the first session, followed by PBMT alone 48 h later in the second session. In the research conducted by Ramalho et al.[22], only aPDT was utilized in a single treatment session. Similarly, the results contrast with the Ajmal et al.[44] study, which applied a single session of aPDT and did not observe a statistically significant difference in pain scores between the aPDT group and the acyclovir group immediately after treatment. However, the analgesic effect of the combination of aPDT and PBMT was similar to that of PBMT alone, with no significant differences between the two groups. Individuals with herpes labialis were categorized into three groups. Group I received 5% topical acyclovir treatment, with participants instructed to apply the cream to the lesion four times daily for 7 days. Group II underwent aPDT, while Group III received 5% topical acyclovir therapy in conjunction with aPDT. The laser used for treatment operated at a wavelength of 660 nm, with power and energy density parameters set at 150 mW and 300 J/cm², respectively. The energy output was measured at 4.5 J, utilizing a spot size of 0.028 cm², with irradiation points arranged 1 cm apart[44]. The present findings align with the study conducted by Seyyedi et al.[2], which indicated that PBMT could serve as a substitute for traditional pharmacological treatments in patients with RHL because of its greater ability to minimize postoperative pain and lesion size and enhance patient satisfaction. The patients were randomly divided into two groups: one group received 5% acyclovir cream with a wavelength of 940 ± 10 nm, an energy density of 4 J/cm², and an output power of 100 mW. The other group received 5% acyclovir five times over five days along with a sham laser treatment[2]. The current study demonstrated that aPDT effectively speeds up the healing of RHL lesions, as we noted that the crusts of the lesions sloughed off more quickly and exhibited a statistically significant difference in patients treated with this method compared to those using acyclovir cream. This finding contrasts with that of Ramalho et al.[22], who did not observe a notable difference in the healing rate between aPDT and acyclovir cream. Several factors could account for this discrepancy. In addition to variations in the laser settings utilized, the inclusion of a second treatment session in our study where we administered PBMT significantly contributed to enhanced healing, as the application of PBMT following aPDT during the crust stage promotes a more organized stimulation of collagen fibers, thereby accelerating the healing process. It's important to highlight that the radiant exposure applied during the second session of LLLT (4.7 J/cm2) was lower than the exposure used in the first session of aPDT (24 J/cm2). This difference is due to the primary aim at this point being to promote the healing process and stimulate the formation of collagen fibers rather than activating the photosensitizer and suppressing the virus[45]. The result aligns with the study of Mello et al.[46]. aPDT was utilized in Group 1 (immunocompetent) and Group 3 (oncologic) using methylene blue 0.01%, followed by 660 nm low-level LASER applied to the affected area. In Group 2, immunocompetent patients were treated with

a prescription for Acyclovir cream 50 mg/g. aPDT is an effective supplementary treatment for HSV infections in the oral and perioral regions for both immunocompetent and oncologic patients[46]. According to Marotti et al.[47], aPDT was utilized as an adjunctive treatment for herpes labialis in four individuals. A specific formulation of 0.01% (m/V) methylene blue solution was administered at the vesicular stage of the herpes viral infection, followed by irradiation of the lesions with laser energy (wavelength 660 nm, energy density 120 J/cm², output power of 40 mW, 2 min per point, delivering 4.8 J of energy per point, at four locations). After 24 h, the patients returned for a second round of phototherapy using the same equipment, this time applying 3.8 J/cm² with a power output of 15 mW, achieving a total dose of 0.6 J. This same method was repeated after 72 h and again one week later. The application of aPDT for treating herpes labialis was successful, exhibited no adverse effects, and when combined with laser phototherapy, it expedited the healing process[47].

The main limitation of the study is that participants were not monitored for a long enough period to assess the impact of treatment on relapse. However, according to earlier research, the combination of aPDT and PBMT seems to contribute to a decrease in relapse, even though the underlying mechanism remains unclear[47–49]. Some research has linked this effect to the capacity of aPDT to trigger an acute inflammatory response, which activates the innate immune system to produce cytotoxic T cells that can recognize and destroy virus-infected cells, releasing further mediators in the process [52]. On the other hand, other studies have suggested that PBMT's role in influencing the immune system response is responsible for reduced herpes recurrence[35]. Regardless of which treatment alters the immune system's response, both therapies seem to significantly aid in lowering relapse rates, and additional research with extended follow-up is necessary to validate these findings. Moreover, it is recommended to conduct future research involving a group specifically targeted at aPTD and to repeat the administration of aPTD or PBMT along with longer follow-up periods. In addition, including measures of patient satisfaction, quality of life, and systematic tracking of side effects in future research.

Conclusions

In the circumstances of this study, the following result has been obtained. The findings indicate that aPDT and PBMT offer a promising approach to treating RHL. This treatment method outperforms traditional acyclovir cream and PBMT, improving its efficacy in speeding up recovery and alleviating symptoms.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

M.A.G.A.H. Carried out the experiment, and drafted the manuscript. M.K. Performed the statistical analysis, and wrote the manuscript. J.A.H. Supervised the project. A.A.A. Planned the experiments, supervised the project, and critically reviewed the manuscript. All authors have read and approved the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Informed consent

Informed consent was obtained from all subjects.

Additional information

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