Original Article

The Effect Of Combined 650 Nm And Infrared Laser On Chronic Diabetic Foot Ulcer Surface Area: A Randomized Controlled Trial

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Abstract

Background Photobiomodulation PBM therapy reduces inflammation and oxidative stress, and accelerates cell migration and proliferation, release of key growth factors, thereby improving tissue regeneration and accelerating wound healing. **Objective** to investigate the effect of combined 650 nm and infrared laser on chronic diabetic foot ulcer healing. **Methods** Forty-five patients with an age range from 18-60 years old, both sexes, were assigned randomly into three equal groups, each group consisting of 15 patients, group A received laser therapy in sequential mode, group B received laser therapy in separate mode and the control group C receive traditional wound care: the primary outcomes were the wound surface area measurement, percentage of wound complete closure and percentage of days needed for surface area reduction before and after receiving the treatment protocol for two consecutive months, and the secondary outcomes were % of wound cause and location frequencies. **Results** The results showed a statistically significant reduction in wound surface area within groups A & B ($P < 0.05$) while there was no statistically significant improvement of WSA in group C. Comparison between groups showed a statistically significant difference between mean values of WSA measured between study groups after treatment (p-value = 0.003) with lower values at group A & B in comparison to group C (p-values = 0.002 & 0.008 respectively) and no significant difference between group A & B (p-value = 0.361). **Conclusion** These findings suggest that group A is favored over group B in accelerating the wound-healing process of diabetic foot ulcers.
Trial registration ClinicalTrials.gov Identifier ID-no.: NCT05517863

Keywords: infrared laser, chronic diabetic foot ulcer, wound surface area.

INTRODUCTION

Diabetes mellitus (DM) is considered a major public health concern across the world, and its prevalence is rising, especially in developing countries [**1**]. Globally, adults with diabetes accounted for 463 million in 2019, and this figure is anticipated to rise to 642 million by 2040. According to the International Diabetes Federation, Egypt is one of the ten countries with the greatest prevalence of diabetes. The number of diabetic patients in Egypt is likely to rise from 9 million in 2019 to 13.1 million by 2035 [**2**].
In Egypt, the frequency of DFUs is significant, ranging from 6.1% to 29.3% [**3**]. About half of the DFUs will be infected across their lifetime [**4**]. This infection starts superficially, but if the therapy is delayed and immunity is compromised, it can spread to the deeper tissues and cause gangrene and amputations [**5**].
DFUs have a complicated etiology, to which several factors contribute including socio-demographic factors like age, gender, residence, and educational status [**6**]; clinical factors such as duration and type of DM, poor glycemic control,

increased body mass index (BMI), and foot deformities. Comorbidities including peripheral vascular disease (PVD), retinopathy, nephropathy, and neuropathy are also associated with an increased risk of developing DFUs [**7**].

Factors responsible for the delayed wound healing observed in the diabetic foot include vascular disease and neuropathy as discussed above. However, several other molecular factors are equally involved in this phenomenon. Wound angiogenesis is defective in diabetes. In the process of wound healing, a balance is achieved between excess and deficient angiogenesis, and this is apparently altered in diabetes [**8**]. In diabetic wounds, however, there is deficient angiogenesis based on the observation of decreased vascularity and delayed closure time [**9**].

Most of the study findings stated that laser therapy, or low-level laser therapy (LLLT), modulates the expression of inflammatory mediators and leads to a reduction in edema, leukocyte influx, and oxidative stress [**10**]. Further, LLLT has been shown to stimulate neo-vascularization and collagen remodeling16 to heal wounds in a faster way. Few of the studies show that a 660 nm wavelength shows more viability than a longer wavelength [**11**].

High-intensity laser therapy HILT is considered an effective safe modality for wound healing in patients with foot ulcers, laser therapy also can improve local blood circulation, blood vessel permeability, and cell metabolism [**12**]. The 630–700 nm wavelengths are used to improve the blood supply, stimulate the metabolism of skin cells, and accelerate wound healing; and the 800–1,200 nm wavelength is used to accelerate wound healing and support the treatment of ulcerations [**13**].

Methods

Study Design/Control

This study is designed to be a Randomized control trial RCT, either type one or type two diabetic patients with grade I and II 2 chronic diabetic foot ulcers according to Wagner classification. This study was conducted on diabetic patients who were recruited from vascular surgery departments in the kasr Aini hospital. Figure (1) shows the number of patients recruited and included in the study.

Figure (1) CONSORT flow chart for the numbers of patients included in the three groups.

The forty-five patients will be assigned randomly into three equal groups, each group consisting of 15 patients:

Group (A) received laser therapy with 4 wavelengths in a synchronized mode in one beam plus traditional wound care treatment in form of (debridement, irrigation with saline, daily dressing) & foot care, and wear.

Group (B) received laser therapy with 4 wavelengths in separate modes plus traditional wound care treatment in form of (debridement, irrigation with saline, daily dressing) & & foot care, and wear.

Group (C) received traditional wound care treatment informs of (debridement, irrigation with saline, daily dressing) & foot care and wear.

Inclusion criteria

1. Diabetic patients with either type I or II.
2. Age from 18-60 years old, both sexes.
3. Ulcer lasting longer than two months.
4. DFU grade 1 or 2 according to the Wagner classification.
5. All patients are able to walk independently.

Exclusion criteria

1. Patients with vascular disease (arterial or venous).
2. Patients with fixed ankle deformity as Charcot foot or stiffness.
3. Patients with any type of osteomyelitis associated with DFU.
4. Patients with renal or hepatic failure.
5. BMI < 30 kg/m^2 as Obesity can cause poor perfusion due to vascular insufficiencies; an altered population of immune mediators may lengthen the inflammatory process & decrease oxygenation of subcutaneous adipose tissue which is liable to be infected.

Clinical assessment

a) Demographic data

1. Age.
2. Sex.
3. BMI.

b) Wound measurement data

1. Percentage of wound size measurement methods.
   a. A ruler (wound area was calculated by measuring and multiplying the greatest length by the greatest width perpendicular to the greatest length).
   b. A sterile transparent film sheet.
2. Percentage of wounds complete closure.
3. Percentage of days needed for surface area reduction.
4. Percentage of each type of wound caused.
5. Percentage of each wound location.

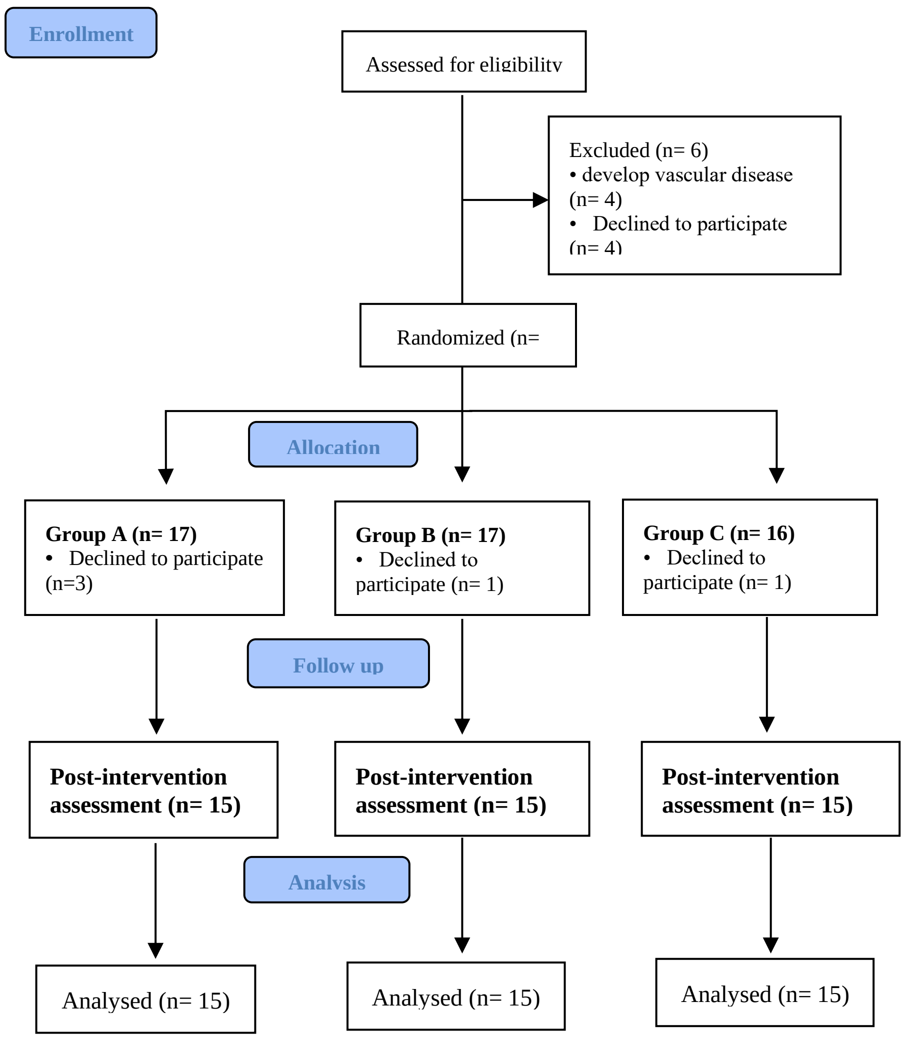

Figure 1. CONSORT flow chart for the numbers of patients included in the three groups.

Treatment protocol

Group (A)

All patients received 2 sessions of laser therapy/week in two consecutive months of treatment aiming for complete wound closure**,** patients received & infrared laser therapy plus traditional wound care:
Use red & infrared laser therapy devices with 4 different wavelengths in a synchronized mode:

1. 980 nm for wound decontamination, improve circulation, and lymphatic drainage.
2. 915 nm enhances O_2 delivery.

3. 810 nm increases ATP production.
4. 650 nm accelerates surface healing and tissue regeneration.

Laser device used

Summus, platinum P4, class laser therapy Diode laser 4 wavelengths (980, 915, 810, and 650), peak power up to 24 watts & frequency up to 20 kHz, Country of made in the USA. Summus device is the 1st laser device that delivers a combination of 4 wavelengths in the same beam at six phases in the same session with auto parameter calculation for synchronization of 4 wavelengths according to wound size to fulfill all laser tissue interaction for proper wound healing.

In the current study we use the following average parameters during the treatment protocol:

1. Average power output: 4 W.
2. Fluence: 4- 10 J/cm^2.
3. Mode: pulsed to deliver high laser energy to deeper tissues mitigating superficial heat build-up.
4. Handpiece radius = 5 cm.
5. Spot size (area) = 7.5 cm^2.
6. Application:
   a. Non-contact laser exposure technique not spotting to avoid the extra thermal effect.
   b. Non-contact technique as it is an open wound to prevent infection with a distance between lenses & wound not exceeding 1 inch.

Traditional wound care informs

1. Wound care treatment
   a. Debridement to remove necrotic tissue.
   b. Irrigation of the wound by normal saline.
   c. Change dressing daily to protect the wound from infection.
2. Foot care
   a. Wash feet daily and dry them carefully, especially between the toes.
   b. Avoid extreme temperatures.
   c. Inspection daily of foot blisters.
3. Footwear
   a. Avoid walking barefoot.
   b. Properly fitted shoes.
   c. Avoid wearing open-toed shoes.

Results

The final statistical analysis included 45 patients, 15 in each group. The Chi-Square test showed no statistically significant difference between groups regarding frequencies of the cause of wound onset, wound location, and sex. At baseline (Table 1, and Figure 2), a statistically one-way ANOVA test showed there was no significant difference between study groups (A and B) regarding age, BMI, and WSA pretreatment.

WSA measurements

The within-group comparison showed a statistically significant reduction in wound surface area within sequential and separate groups ($P < 0.05$) while there was no statistically significant improvement of WSA in the control group after treatment in comparison to before-treatment values. Comparison between groups showed a statistically significant difference between mean values of WSA measured between study groups after treatment (p-value = 0.003) with lower values at sequential and separate groups in comparison to control (p-values = 0.002 & 0.008 respectively) and no significant difference between sequential and separate groups (p-value = 0.361) (Table 1).

Table (1) Comparison between (Mean ± SD) values of outcome measured variables pre- and post-treatment within and between groups.

Parameter	Level	Sequential group (n= 15) Mean ± SD	Separate group (n= 15) Mean ± SD	Control group (n= 15) Mean ± SD	F statistics P-value (between groups)
Age	At baseline	48.54 ± 7.45	48.09 ± 7.7	48.18 ± 7.53	0.816
BMI	At baseline	23.85 ± 3.36	25 ± 4.43	24.09 ± 3.45	0.966
Days of recovery	At baseline	47.46 ± 12.59	56.91 ± 5.38	59.18 ± 2.71	0.020**
WSA	Before treatment	19.9 ± 8.92	15.95 ± 6.1	12.79 ± 6.58	0.179
	After treatment	2.17 ± 3.05	2.85 ± 2.8	9.76 ± 6	0.003**
	P-value	0.001**	0.003**	0.114	------

** Significant at 0.05.

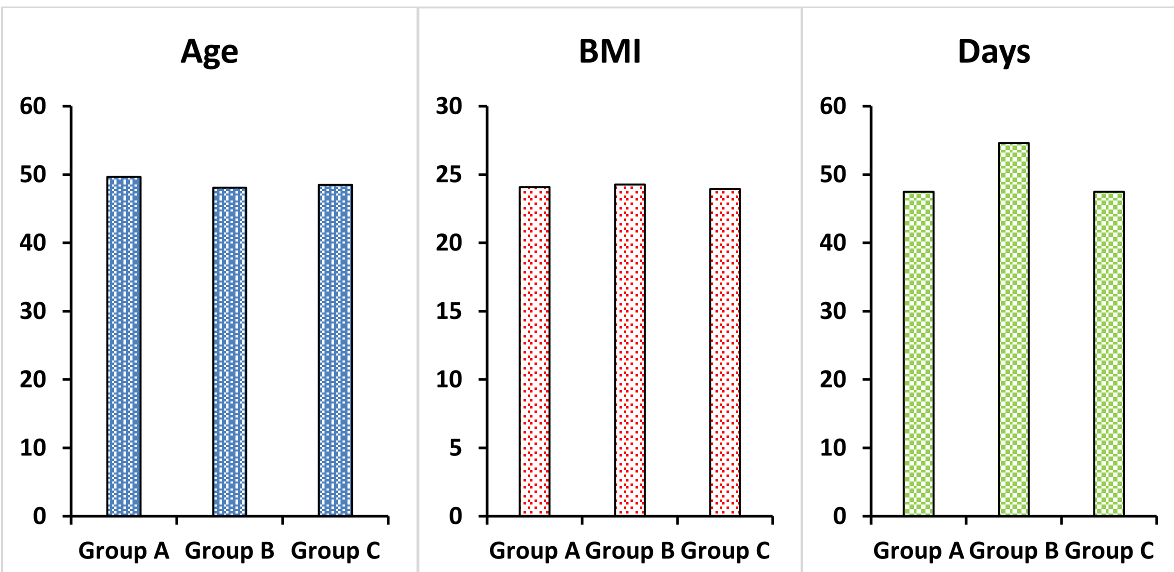

Figure (2) Mean values of general characteristics between study groups.

Days of recovery showed a statistically significant difference between sequential and control groups (p-value = 0.041) with no statistically significant difference between separate and control or sequential and separate groups (p-values > 0.05) (Table 1). Furthermore, a Comparison between percentages of closed wounds after 2 months in the 3 study groups showed a significantly higher closure percentage in Group A (Table 2).

Table (2) Pairwise comparison between test groups regarding Days of recovery and WAS.

Groups	**Group type**	**WSA after treatment (p-value)**	**Days of recovery (p-value)**
Sequential group	Separate group	0.361	0.093
Sequential group	Control	0.002**	0.041**
Separate group	Control	0.008**	0.438

** Significant at 0.05.

The frequencies and the percent of change within each group of non-menial variables between groups show a non-significant difference in different causes of wound onset, and also a non-significant difference between different wound locations, and last no significant difference between different gender (males and females). But there is a significant difference in the number of cases with complete wound closure in the sequential laser mode group more than in the separate laser mode group as shown in Table 3, and Figure 3.

Table 3. Comparison between (frequency (% within each group) of non-menial variables between groups.

Parameters	Type/ Location	Group A	Group B	Group C	P-value
Cause of wound onset	Clean cut	1 (6.7%)	2 (13.3%)	1 (6.7%)	0.968
	Footwear	5 (33.3%)	7 (46.7%)	5 (33.3%)	
	Needle	1 (6.7%)	2 (13.3%)	1 (6.7%)	
	Needle	1 (6.7%)	0 (0.0%)	1 (6.7%)	
	Trauma	5 (33.3%)	3 (20.0%)	5 (33.3%)	
	Unknown	2 (13.3%)	1 (6.7%)	2 (13.3%)	
Wound location	Dorsal	0 (0.0%)	2 (13.3%)	0 (0.0%)	0.420
	Heel	4 (26.7%)	3 (20.0%)	4 (26.7%)	
	Lateral	0 (0.0%)	2 (13.3%)	0 (0.0%)	
	Lateral	2 (13.3%)	1 (6.7%)	2 (13.3%)	
	Planter	5 (33.3%)	2 (13.3%)	5 (33.3%)	
	Toe	4 (26.7%)	5 (33.3%)	4 (26.7%)	
Wound closure	Closed	9 (60.0%)	4 (26.7%)	1 (6.7%)	0.006*
	Open	6 (40.0%)	11 (73.3%)	14 (93.3%)	
Sex	F	7 (46.7%)	7 (46.7%)	9 (60.0%)	0.701
	M	8 (53.3%)	8 (53.3%)	6 (40.0%)	

P-value calculated by Chi-Square test.
* Significant at 0.05.

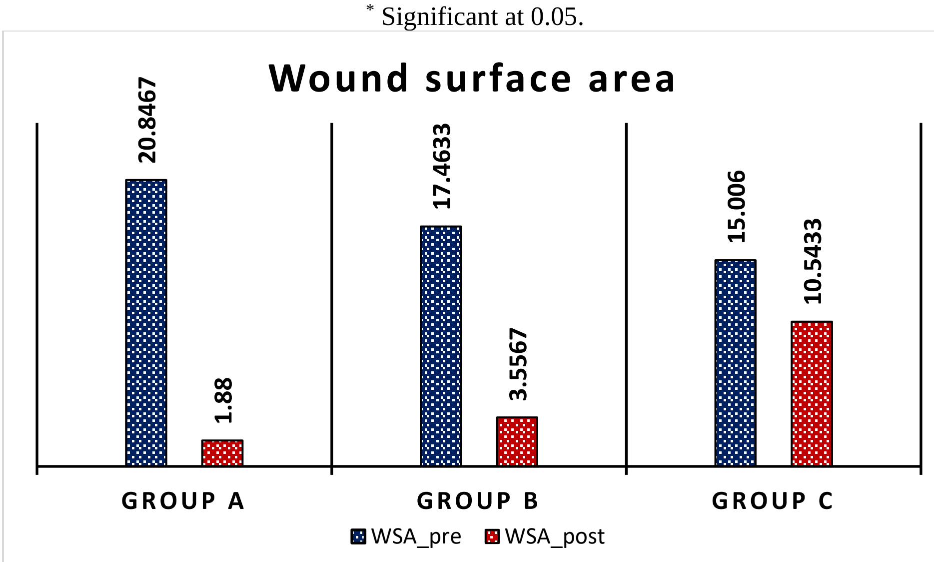

Figure 3. Mean values of WSA before and after treatment in study groups.

Discussion

Effect of laser on Inflammation & ROS production

Reactive oxygen species (ROS) are massively generated during the early phases of OM and play a major role in the pathogenesis of inflammation in general. We found that the various wavelengths differentially modulate ROS production [**14**]. In particular, the 660nm laser light increases ROS production when applied either before or after an oxidative stimulus. In contrast, the 970 nm laser light exerted a moderate antioxidant activity both in the saliva of OM patients and in both cell types. The most marked reduction in the levels of ROS was detected in cells exposed either to the 800 nm laser light or to the combination of the three wavelengths [**15**]. Overall, our study demonstrates that PBM exerts different effects on the redox state of both PMNs and keratinocytes depending on the used wavelength and prompts the validation of a multi-wavelength protocol in clinical settings. To understand whether the same effects are also exerted on keratinocytes, we exploited genetically encoded redox biosensors to monitor ROS production in real-time in response to PBM, this time also testing an additional wavelength (800 nm), which is being progressively used to reach inflamed tissues in depth, as this wavelength is poorly absorbed by water. We confirmed that the 660nm laser light increases ROS production when applied either before or after an oxidative stimulus [**16**]. More importantly, we found that the 970nm laser light exerted moderate antioxidant activity, whereas a striking reduction in the levels of ROS was detected in cells exposed to either the 800nm laser light or the combination of the three different wavelengths [**14**].

Effect of laser on ATP production

PBM stimulates the mitochondria increasing adenosine triphosphate (ATP) production, the main source of energy source for the majority of cellular functions, increasing the cells' ability to fight infection and accelerate healing. And the downstream release of growth factors [**17**]. The binding of growth factors to cell surface receptors induces signaling pathways that transmit signals to the nucleus for the transcription of genes for increased cellular proliferation, viability, and migration in numerous cell types, including stem cells and fibroblasts. Tissues exposed to PBM absorb the specific wavelength of light through the mitochondrial respiratory chain enzyme, cytochrome C oxidase (COX). COX, together with porphyrins and heme proteins, plays a crucial role in the absorption of light. The action of COX is maximized at wavelengths of 580–700 nm. COX absorbs photon energy (light), which initiates photochemical and photophysical cascades, stimulating the production of ATP [**18**].

Effect of laser on circulation & lymphatic drainage

Free Nitric Oxide (NO) & ATP & Reactive Oxygen Species (ROS), Enzymes (like Beta Endorphin) and enhances Blood Flow and Lymphatic Drainage improving blood circulation. The release of NO, a potent Vasodilator increase circulation, decreases inflammation, and enhances the transport of oxygen and immune cells through the tissue [**19**]. The increase in the level of NO might have caused an important effect on angiogenesis as NO is known to influence both angiogenesis and neovascularization. Topical application of various kinds of low-level laser therapy (LLLT), mainly in continuous mode of the red spectrum (633-635 nm) and in the pulsed mode of infrared (IR) spectrum (890-904 nm) when treating trophic ulcer [**20**]. The abilities of LLLT non-invasive irradiation to restore the elasticity of the cell membranes, to normalize lymph and hemo microcirculation in the affected area, and to restore the regulating functions of tissues, organs, and the entire body by activating enzyme systems and metabolism, are very useful in the treatment of patients with the venous trophic ulcer [**21**].

Combination of red and infrared lasers

In wavelengths of red, this reaction occurs in mitochondria and lysosomes, and in infrared wavelengths, it starts in the cytoplasmic membrane, but both occur to increase the production of ATP in the intracellular environment, enabling the opening of calcium channels in the cytoplasmic membrane and mitochondrial with energy stimulus generated by the cytochrome C oxidase, enabling an increase in the production of RNA by DNA who also suffers increase, all these events are essential for tissue repairs, such as collagen synthesis, the release of histamine by the mast and cell mitosis [**22**]. The additional stimulus of corticosteroids released by the adrenal glands during laser therapy helps to reduce pro-inflammatory cytokines, contributing to the reduction of the phagocytic activity of macrophages [**23**].

It was also found that wound repair was higher when laser therapy was adjusted with higher power intensity and shorter wavelengths which gives the advantage of using class IV laser therapy over class IIIb. But the increase in application frequency from three to five weekly sessions did not result in changes in wound repair, but higher frequencies can inhibit the repair of these lesions [**24**].

Some wavelengths present positive photobiological effects on the healing process. Besides quickening the process, laser light affects the tissue at cellular levels, including a photo-activated increase in cell metabolism. In addition, it also causes analgesia and increases the rate of both inflammatory response and wound healing [**25**].

Conclusion

The red and infrared lasers in general are efficient collaborators in the repair of skin wounds, inducing growth of fibroblasts, collagen synthesis, angiogenesis, and subsequent re-epithelialization to wound closure. the study found a promising alternative for the use of lasers to achieve these results is the combination of red and infrared wavelengths as in sequential mode in the percent of wound closure and also the number of days needed for complete wound closure in two consecutive months of treatment compared to the application of separate mode as red laser activate the photoreceptors that absorb the infrared lasers and produce more ATP needed for wound repair.

Credit authorship contribution statement

Conceptualization; HFA, MSB
Data curation; HFA, MSB
Formal analysis; HFA, MSB
Funding acquisition; HFA, MSB
Investigation; HFA, MSB, MIM
Methodology; HFA, MSB
Project administration; MSB, MIM
Resources; Software; HFA, MSB
Supervision; MSB, MIM
Validation, HSA
Visualization; HSA
Roles/Writing - original draft; HFA, MSB
Writing - review & editing; HFA, MSB

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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