

# Acute effect of kinesiotherapy and neuromotor electrostimulation on thermal variation in individuals with chronic venous insufficiency

*Efeito agudo da cinesioterapia e da eletroestimulação neuromotora sobre a variação térmica de indivíduos com insuficiência venosa crônica*

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## Abstract

**Introduction:** Chronic venous insufficiency (CVI) is a change in the venous system that can be caused by dysfunction in the triceps surae muscles. **Objective:** To evaluate the acute effect of neuromuscular electrical stimulation and kinesiotherapy on ankle joint flexibility, infrared radiation and triceps surae strength in individuals with CVI. **Methods:** We conducted a comparative study, in which ankle flexibility, dorsiflexion and plantar flexion strength and thermal variations of the calf were evaluated. An electrical stimulation session (1 kHz Aussie current with burst = 2 ms) was performed on the right lower limb (EG - electrostimulation group) and kinesiotherapy on the lower limb left (KG - kinesiotherapy group), with stretching and metabolic exercises involving the ankle. **Results:** Nineteen female patients were evaluated. Analysis of ankle flexibility did not demonstrate significant changes. In the assessment of muscle strength, there was no difference between groups, and in the intragroup assessment, only KG showed an increase for dorsiflexion (before treatment:  $11.6 \pm 3.5$ ; 24 h after:  $13.5 \pm 3.0$ ;  $p = 0.02$ ), and for plantar flexion (before:  $11.8 \pm 6.3$ ; 24 h later:  $14.4 \pm 5.06$ ;  $p = 0.04$ ). Regarding thermography, there was no intragroup difference, while in the intergroup assessment, KG showed an increase in temperature immediately and 24 h later ( $0.44 \pm 0.68$ ,  $p = 0.01$  and  $0.25 \pm 0.83$ ,  $p = 0.07$ , respectively). When analyzing the correlation between dorsiflexion and plantar flexion strength of both lower limbs with total range of motion of the right and left ankle, a positive correlation was observed only between the plantar flexion strength immediately after and 24 h later with range of motion and ankle in KG ( $r = 0.49$ ,  $p = 0.03$  and  $r = 0.51$ ,  $p = 0.03$ , respectively). **Conclusion:** There were no significant differences between kinesiotherapy and electrotherapy when analyzing joint flexibility and muscle strength, but kinesiotherapy was superior in increasing calf temperature before and after 24 h of intervention.

**Keywords:** Exercise. Physical therapy. Thermography. Venous insufficiency. Women.

## Resumo

**Introdução:** A insuficiência venosa crônica (IVC) é uma alteração no sistema venoso que pode ser causada por disfunção na musculatura do tríceps sural. **Objetivo:** Avaliar o efeito agudo da eletroestimulação neuromuscular e da cinesioterapia sobre a flexibilidade articular do tornozelo, radiação infravermelha e força do tríceps sural de indivíduos com IVC. **Métodos:** Trata-se de um estudo comparativo, no qual foram avaliadas a flexibilidade do tornozelo, força de dorsiflexão e flexão plantar e variações térmicas da panturrilha. Realizou-se uma sessão de eletroestimulação (corrente Aussie de 1kHz, burst = 2ms) no membro inferior direito (GE - grupo eletroestimulação) e cinesioterapia no membro inferior esquerdo (GC - grupo cinesioterapia), com alongamentos e exercícios metabólicos envolvendo o tornozelo. **Resultados:** Foram avaliados 19 pacientes do sexo feminino. A análise da flexibilidade do tornozelo não demonstrou alterações significantes. Na avaliação da força muscular não houve diferença intergrupos e na avaliação intragrupos apenas o GC apresentou aumento para dorsiflexão (antes do tratamento:  $11,6 \pm 3,5$ ; 24h após:  $13,5 \pm 3,0$ ;  $p = 0,02$ ), e para flexão plantar (antes:  $11,8 \pm 6,3$ ; 24h após:  $14,4 \pm 5,06$ ;  $p = 0,04$ ). Em relação à termografia, não houve diferença intragrupo, enquanto na avaliação intergrupo o GC apresentou aumento da temperatura imediatamente e 24h após ( $0,44 \pm 0,68$ ,  $p = 0,01$  e  $0,25 \pm 0,83$ ,  $p = 0,07$ , respectivamente). Quando analisada a correlação entre força de dorsiflexão e flexão plantar de ambos os membros inferiores com a amplitude do arco total do tornozelo direito e esquerdo, observou-se correlação positiva apenas entre a força de flexão plantar imediatamente após e 24h após com com amplitude de movimento e tornozelo no GC ( $r = 0,49$ ,  $p = 0,03$  e  $r = 0,51$ ,  $p = 0,03$ , respectivamente). **Conclusão:** Não houve diferenças significantes entre a cinesioterapia e a eletroterapia quando analisada a flexibilidade e força muscular, porém a cinesioterapia foi superior no incremento de temperatura da panturrilha antes e após 24h da intervenção.

**Palavras-chaves:** Exercício físico. Fisioterapia. Termografia. Insuficiência venosa. Mulheres.

## Introduction

Chronic venous insufficiency (CVI) is a disease that is present in 35.4% of the Brazilian population, at different levels of classification.<sup>1,2</sup> CVI can be defined as an alteration in the functioning of the venous system

caused by an inability of the valve complex, generating reflux that may or may not be associated with obstruction of venous flow. Furthermore, it may be caused by dysfunction in the triceps surae muscles.<sup>3</sup> The cause of valve malfunction still has gaps in the current literature. It is known that reflux is associated with 90% of CVI cases and may be exacerbated by dysfunction of the triceps surae muscles.<sup>4</sup>

CVI is a highly prevalent disease worldwide, and early diagnosis is important for successful treatment. Diagnosis begins through the individual's clinic, doing an anamnesis and physical examination, considering complaints and duration of symptoms. Furthermore, complementary examinations can be used to determine the location and morphology of changes, such as continuous wave doppler or venous echodoppler, but these are high-cost diagnostic methods.<sup>5</sup> Skin thermography, therefore, can be used as a diagnosis. Complementary, non-invasive, low-cost and painless treatment of physiological dysfunctions of local blood circulation present in CVI.<sup>6,7</sup>

Of the various treatments that can be addressed for CVI, physiotherapy is highlighted for its non-invasive and preventive nature. The resources used to treat CVI range from elastocompression to preventive myolympokinetic exercises and neuromuscular electrostimulation in impulse-aspiration pumps such as that of the triceps surae muscles.<sup>8-11</sup> The current literature suggests improvements in the CVI condition through exercises that work the calf muscles.<sup>12-16</sup> Another way to stimulate the muscles is through excitomotor currents that cause action potentials in deep and superficial motor units, which can be used to strengthen muscles, improve function and reduce edema and pain.<sup>17</sup> However, it is not clear in the scientific literature whether electrostimulation or kinesiotherapy has any superior effect for improving flexibility, strength and infrared radiation in the acute phase of physiotherapeutic treatment of patients with CVI.

In view of the above, the objective of this study was to determine the acute effect of neuromuscular electrostimulation through Aussie current compared to kinesiotherapy on ankle joint flexibility, infrared radiation and triceps surae strength in individuals with CVI.

## Methods

We conducted a comparative study between the effects on strength, ankle flexibility and infrared irradiation

tion after carrying out a treatment protocol with electrostimulation (EG - electrostimulation group) and a kinesiotherapy protocol (KG - kinesiotherapy group) in individuals with CVI.

The project was approved by the Ethics Committee of the Federal University of Pernambuco (UFPE) under Approval No. 4,835,993. The sample was obtained by convenience from volunteers screened at the angiology outpatient clinic at Hospital das Clínicas, in Recife/PE, considered a highly complex hospital that exclusively serves patients from the Unified Health System (SUS) network.

Patients with CVI were referred to the Multi-User Integrated Analysis Laboratory (LAMAI), in the Department of Physiotherapy at UFPE, where the study was carried out. Patients who agreed to participate in the research signed an informed consent form.

### Elegibility criteria

Female volunteers, over 18 years old, with CVI in both lower limbs with clinical classification (CEAP) grades II, III and IV were included. The CEAP classification describes the severity of chronic venous diseases and involves the analysis of clinical signs (C), etiology (E), anatomy (A) and pathophysiology (P) of the condition. Patients were excluded if they had decompensated diabetes, were pregnant, had neuropathies, acute deep venous thrombosis, chronic obstructive arteriopathy, ulcers of non-venous origin or decompensated hypertension.

### Evaluation methods

Initially, a sociodemographic and clinical assessment of the patients was carried out and then a physical assessment was performed. To evaluate the total range of motion of the ankle, the smartphone app called "goniometer" was used using the measurement in degrees. For measurement, the individual was positioned sitting on a standard-sized stretcher, with their feet out. The movement began with the foot in full plantar flexion, followed by plantar dorsiflexion, avoiding knee extension and any type of hip movement as much as possible to prevent movement compensation.<sup>18,19</sup>

Next, ankle dorsiflexion and plantar flexion strength was assessed using the Hoggan Microfet 2 portable dynamometer. To measure peak strength in dorsiflexion,

the individual was positioned in supine decubitus on a stretcher, with the ankle on a medium-sized pillow to prevent the foot from touching the mattress and hindering movement. Before starting the evaluation, a belt was placed on the dynamometer to stabilize the evaluator's body. Soon after, the MicroFet was positioned on the individual's foot and the evaluator gave verbal stimuli to perform the dorsiflexion movement with as much force as possible without moving the hips or knees.

To measure peak plantar flexion strength, the individual was placed in the prone position with both feet outside the stretcher. The Microfet was positioned under the arch of the foot, seeking the greatest possible contact between the Microfet and the foot. A cushion was placed between the Microfet and the wall to make movement more comfortable. The evaluator gave verbal stimuli to the participants to perform the plantar flexion movement with as much force as they could without moving their hips or taking their feet off the equipment.

Each of the procedures was done four times, where the first time was just to be familiarized with the movement. For analysis purposes, the peak strength of the last three measurements was recorded using the arithmetic mean. The assessment was carried out before the physiotherapeutic protocols, immediately after and 24 h after the interventions.

The assessment of infrared radiation was obtained from thermal image capture procedures (thermograms) carried out in accordance with the recommendations of the European Association of Thermology.<sup>20,21</sup> Individuals were instructed not to perform vigorous physical activity in the 24 h prior to the exam, not to consume alcohol or caffeine and not to use any type of cream or lotion on the skin in the six hours preceding the evaluation. Before the thermographic evaluation, a period of 10 min of acclimatization was respected in which the volunteers had the region to be evaluated naked.<sup>21</sup> After acclimatization, the volunteers were in an anatomical position and the camera remained stabilized on a tripod, 1.5 m from each subject, with the lens positioned perpendicular to the region of interest (ROI).

To analyze the thermograms in the posterior view, the delimitations of the ROIs were considered by adopting the anatomical points of the popliteal line and Achilles tendon, while in the lateral view the lower end of the lateral malleolus was considered. The analyses were carried out with the values obtained from skin

thermometry, with comparisons (delta) being made between contralateral ROIs to determine the level of attention to asymmetry. The delimitations and qualitative and quantitative analyses of the ROIs were carried out using Thermofy software.

### Interventions

After the assessment procedures, interventions were carried out: for the right lower limb (RLL), the electrostimulation protocol was carried out and then the kinesiotherapy protocol on the left lower limb (LLL). After executing the protocols, the individual rested for five minutes before being placed back in a bipedal position for a new image capture with the thermographic camera, assessing flexibility and muscle strength.

After 24 h of interventions, reassessments of the same variables were carried out. The researcher who carried out the initial and final assessments was blind to the interventions and the physiotherapist who carried out the intervention was blind to the assessment procedures.

Electrostimulation was performed in a single intervention, using Aussie current, with volunteers positioned in the prone position on a stretcher. Asepsis was performed using cotton wool and 70% alcohol in the treated region. A 10-channel Neurodyn device (IBRAMED) was used, in which the application took place four fingers below the popliteal line and four fingers above the beginning of the Achilles tendon, totaling the use of two rubber electrodes parallel to each other. The electrostimulation parameters used were a frequency of 1 kHz with a burst duration of 2 ms. The burst modulation frequency was 50 Hz; rise: 3 s; on: 6 s; decay: 3 s; off: 12 s. The current application time was 20 min.

The stimulation intensity was that which caused comfortable but visible muscle contraction, increasing the intensity according to the volunteer's sensitivity. The intervention lasted around 35 min, considering the initial and final rest time for thermography capture.<sup>22</sup>

Kinesiotherapy was also performed in a single session, lasting approximately 30 min and consisted of: stretching exercises for the knee flexors and extensors, thigh adductors and abductors; metabolic exercises of ankle flexion-extension and encirclement, hip flexion with ankle flexion-extension; and resistance exercises with an elastic band, with the calf in the orthostatic position, as recommended by Leal et al.<sup>23</sup>

### Statistical analysis of data

Data analysis was performed using GraphPad Prism software (USA). Continuous data are reported as means  $\pm$  standard deviation, while absolute data are reported as total value and percentage. Normality was verified by the Shapiro-Wilk test. The repeated measures ANOVA test, Tukey post-test and paired and unpaired t-test were used to evaluate the differences between intra- and intergroup means. Pearson correlation was used, considering reference values ( $r$ ) 1 to -1, with  $r > 0.8$  = excellent correlation; 0.6 - 0.8 = moderate correlation; 0.3 - 0.5 = weak correlation; and  $r < 0.3$  = no correlation. Negative values reproduce inversely proportional correlation, while positive values reproduce correlation directly. All tests were two-tailed, and in all statistical analyses, a significance level of  $p \leq 0.05$  was adopted.

### Results

Nineteen patients who had CVI in both legs were evaluated. The sociodemographic and clinical characterization of the sample is presented in Table 1. The mean age was  $60 \pm 7.9$  years, weight  $71.1 \pm 11$  kg, height  $1.57 \pm 0.1$  cm and body mass index (BMI)  $28.9 \pm 4.2$ .

In the assessment of muscle strength, there was no statistically significant difference between groups. In the intragroup evaluation, only KG showed an increase in strength for dorsiflexion when comparing the moments before treatment and 24 h after. For plantar flexion, an increase in strength was observed in the same group when comparing the moments before treatment and 24 h after treatment (Table 2). The degree of mobility showed no difference between or within groups at the moments evaluated (Table 3). Regarding thermography, a significant intergroup difference was found, characterized by distinct effects between groups. In KG, the temperature increased immediately after treatment and maintained the increase after 24 h, while EG showed decreased temperature immediately after the treatment. In other words, KG seems to bring a greater thermal change in relation to the pre-treatment image (Table 4).

Table 5 presents the correlation between dorsiflexion and plantar flexion strength of both lower limbs with the total range of motion of the right and left ankle. A positive correlation was observed only between the plantar flexion strength immediately after and 24 h later with the ankle range of motion in KG.

**Table 1** - Sociodemographic and clinical characterization of individuals with chronic venous insufficiency (n = 19)

Variable	n (%)
Sex, female	19 (100)
<b>Marital status</b>	
Single	6 (31.6)
Married	8 (42.1)
Widowed	2 (10.5)
Divorced	3 (15.8)
<b>Education level</b>	
Secondary, complete	9 (47.4)
Secondary, incomplete	1 (5.2)
Primary, complete	3 (15.8)
Primary, incomplete	4 (21.1)
Technical, complete	2 (10.5)
<b>City of origin</b>	
Recife	6 (31.6)
Jaboatão dos Guararapes	13 (68.4)
<b>Treated with physical therapy</b>	
Yes	10 (52.6)
No	9 (47.4)
<b>Allergic</b>	
Yes	5 (26.3)
No	14 (73.7)
<b>Smoker</b>	
Yes	0 (0.0)
No	19 (100)
<b>Drinks alcohol</b>	
Yes	6 (31.6)
No	13 (68.4)
<b>Diabetes</b>	
Yes	7 (36.8)
No	12 (63.2)
<b>Hypertension</b>	
Yes	12 (63.2)
No	7 (36.8)
<b>Cardiovascular disease</b>	
Yes	0 (0.0)
No	19 (100)
<b>Sedentary</b>	
Yes	9 (47.6)
No	10 (52.6)
<b>Obesity</b>	
Yes	8 (42.1)
No	11 (57.9)
<b>Exercise</b>	
Yes	11 (57.9)
No	8 (42.1)

**Table 2** - Muscle strength (kgf), intra- and intergroup, at three different times (before, immediately after and 24 h after treatments) in individuals with chronic venous insufficiency (n = 19)

	Before	Immediately after	24 h after	p-value
<b>Dorsiflexion</b>				
Electro	13.0 ± 3.9	13.6 ± 3.6	13.5 ± 2.8	0.57*
Kinesio	11.6 ± 3.3	12.8 ± 3.3	13.5 ± 3.0†	0.02†**
p-value	0.25**	0.52**	0.98**	-
<b>Plantar flexion</b>				
Electro	12.6 ± 5.3	14.2 ± 5.7	13.8 ± 5.7	0.33*
Kinesio	11.8 ± 6.3	13.9 ± 6.2†	14.4 ± 5.6	0.04†*
p-value	0.65**	0.89**	0.73**	-

Note: \*Repeated measures ANOVA; \*\*Unpaired t-test; †Difference compared to baseline. Values in bold indicate statistical difference. Electro = electrostimulation; Kinesio = kinesiotherapy.

**Table 3** - Total range of motion of the tibiotarsal joint in degrees (before, immediately after and 24 h after treatments), in individuals with chronic venous insufficiency (n = 19)

Group	Before	Immediately after	24 h after	p-value
Electro	58.6 ± 9.0	59.2 ± 9.6	59.9 ± 6.2	0.71*
Kinesio	59.4 ± 7.7	61.6 ± 8.4	60.7 ± 7.2	0.49*
p-value	0.75**	0.42**	0.71**	-

Note: \*Repeated measures ANOVA; \*\*Unpaired t-test. Electro = electrostimulation; Kinesio = kinesiotherapy.

**Table 4** - Thermal variations ( $\Delta t$ ) before x immediately after and before x 24 h after electrostimulation and kinesiotherapy performed on individuals with chronic venous insufficiency (n = 19)

Lower limb	$\Delta t$ before x immediately after	$\Delta t$ before x 24 h after	p-value
Electro	-0.64 ± 1.76	0.10 ± 1.66	0.17*
Kinesio	0.44 ± 0.68	0.25 ± 0.83	0.36*
p-value	0.01**	0.07**	-

Note: \*Paired t-test; \*\*Unpaired t-test. Values in bold indicate statistical difference. Electro = electrostimulation; Kinesio = kinesiotherapy.

**Table 5** - Correlation between dorsiflexion and plantar flexion strength (kgf) with the total range of motion (ROM) of the ankle immediately after and 24 h after electrostimulation and kinesiotherapy performed on individuals with chronic venous insufficiency (n = 19)

Groups	r (CI)	p-value
Stimulation		
Dorsiflexion strength immediately after x ankle ROM	0.33	0.16
Dorsiflexion strength 24 h after x ankle ROM	0.04	0.84
Plantar flexion strength immediately after x ankle ROM	0.33	0.16
Plantar flexion strength 24 h after x ankle ROM	0.10	0.66
Kinesiotherapy		
Dorsiflexion strength immediately after x ankle ROM	0.29	0.22
Dorsiflexion strength 24 h after x ankle ROM	0.13	0.56
Plantar flexion strength immediately after x ankle ROM	0.49	<b>0.03*</b>
Plantar flexion strength 24 h after x ankle ROM	0.51	<b>0.02*</b>

Note: Pearson's correlation \*p ≤ 0.05. Values in bold indicate statistical significance. CI = confidence interval.

## Discussion

This study compared the effects of electrical stimulation with kinesiotherapy on the outcomes of ankle flexibility, dorsiflexion and plantar flexion strength and thermal variations of the calf before and immediately after the treatment of 19 female patients with CVI, no significant differences were found between the procedures when analyzing ankle flexibility and the strength of the plantar flexor and dorsiflexor muscles. Kinesiotherapy was superior to electrostimulation in increasing calf temperature before and after 24 h of the intervention. In the intragroup analysis, kinesiotherapy produced a significant difference in strength gain in dorsiflexion and plantar flexion immediately and 24 h after the intervention.

Among the factors that can lead to CVI are genetic factors and sex, with the female sex being the most affected.<sup>24-26</sup> In the present study, women with CVI exclusively sought physical therapy treatment. According to the clinical findings in the lower limbs, it can be observed that the most common characteristics present represent the C2 and C3 stages of CVI, according to the CEAP classification, corroborating the study by Santler and Goerge.<sup>4</sup> Individuals with CVI may present with edema, hyperpigmentation, telangiectasia, varicose veins, pain and decreased muscle strength in the lower limb. The acute increase in muscle strength, after the kinesiotherapy protocol in the present study, corrob-

rates the study by Schmidt et al.,<sup>12</sup> which states that physical exercise has a positive impact on muscle strength, resulting in a reduction in muscle strength, capillary fragility and improvement in venous return.

No studies were found that used Aussie current electrostimulation in women with CVI. However, when used in combined therapy treatments, the current is capable of reducing local circumference.<sup>27</sup> Furthermore, the Aussie current alone stimulates the blood, lymphatic and muscle systems.<sup>28</sup> The justification for using this current in this population is that it offers electrical stimulation with minimal discomfort, as it is a medium frequency current and has burst modulation.

No studies were found in the current literature that verified the effects of Aussie current on the gain in muscular strength of the plantar flexors of individuals with CVI. However, Palma et al.<sup>29</sup> used Aussie current with a frequency of 1000 Hz in the quadriceps muscle in patients restricted to bed. The authors did not observe an increase in the cross-sectional area of the rectus femoris, only preservation of strength,<sup>29</sup> which may support the present study, as there was no significant increase in muscle strength in EG.

CVI causes several mobility changes in the body, especially in the lower limb. It is known that the range of motion of the ankle can be impaired, also interfering with the function of the calf muscle pump. In the present study, no significant improvements were found when comparing ankle flexibility after a single session of

kinesiotherapy and electrotherapy. In the study by Schmidt et al.,<sup>12</sup> however, there was an improvement in mobility when comparing the moments before and after the exercise protocol, which consisted of open and closed kinetic chain exercises, through plantar flexion and ankle dorsiflexion. On the other hand, in the study by Schmidt et al.<sup>12</sup> 24 sessions were carried out, while in the present study, there was only a single intervention, suggesting that the desired result cannot be achieved acutely.

Ercan et al.<sup>30</sup> used stability, walking and intermittent pneumatic compression exercises three times a week, for 12 weeks, in patients with CVI, resulting in an improvement in ankle range of motion and ankle muscle strength and a reduction pain, demonstrating the effectiveness of a prolonged and continuous protocol in this population.

CVI alters venous return. One of the effective diagnostic methods for evaluating local blood circulation, chronic pain and vascular changes is skin thermography, which is performed in a painless, quick and non-invasive way.<sup>6</sup> Kelechi et al.<sup>31</sup> compared individuals with and without CVI and observed that the increase in the local temperature could be an indicator for the appearance of ulcerations on the skin and could provide sufficient data early on to initiate an intervention.

The thermal reduction effects observed after the electrostimulation procedure were antagonistic to kinesiotherapy, which demonstrated a thermal increase effect, revealing that kinesiotherapy seems to be more effective for individuals with CVI, since this disease is caused by a dysfunction in the venous system, triggering valve failure or obstruction, which can affect the superficial or deep venous system, together or separately, with negative effects on local circulation. Thus, kinesiotherapy appears to contribute to improving the flow of local circulation in individuals with CVI.<sup>32</sup> The decrease in temperature in EG may have occurred because of a sympathetic vasomotor response, with stimulation of fine fibers that contract the microcirculation. The results obtained by thermography allowed visualization of the effects of both procedures performed and point to an improvement in venous return and a possible reduction in local toxins in patients with CVI.

The recruitment of patients to form a homogeneous sample at baseline and to attract patients to treatment was very limiting, considering that this study was developed during the period of the COVID-19 pandemic.

## Conclusion

There were no significant differences between the kinesiotherapy and electrotherapy procedures when analyzing ankle flexibility and the strength of the plantar flexor and dorsiflexor muscles. Kinesiotherapy presented superior results to electrostimulation in increasing calf temperature before and after 24 h of the intervention.

Since this is the first comparative study that analyzes the effects of Aussie current to a kinesiotherapy protocol in a sample of patients with CVI, it is suggested that the methods used in this study be reproduced in larger samples of patients with characteristics identical to the study current in order to consolidate the results obtained in the present study.

As contributions to the practice of physiotherapy, the use of thermography can be pointed out as a complementary means of diagnosing the severity of the disease and the importance of including kinesiotherapy procedures to increase local calf circulation in patients with CVI.

## Authors' contributions

DLA, APLF, JNM and MAA were responsible for conceptualizing this study. MLB, RLSA, JNM and MAA constructed the study methodology. DLA, RLSA, RSM and VFO participated in data collection. APLF, JNM, MLB, and MAA administered and guided the entire study, and wrote the manuscript. All authors approved the final version of this article.

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