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EFFECT OF PHOTOBIOMODULATION ON HEART RATE VARIABILITY AND INFRARED THERMOGRAPHY OF INDIVIDUALS WITH CEREBRAL PALSY

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ABSTRACT
Introduction: Cerebral palsy (CP) is characterized by a set of non-progressive but mutable disorders, caused by an immature brain lesion and accompanied by imbalances in the sympathetic-vagal balance, with lower heart rate variability (HRV) when compared to individuals with typical development. Infrared thermography (IRT) is able to observe sympathetic activity through skin temperature remotely and non-invasively. Photobiomodulation therapy (BMT) has shown promising effects on modulation responses to autonomic variables. Objective: To verify the effect of BMT-MBT on autonomic variables in individuals with CP. Methods: Eight children and adolescents (8.75 ± 1.67 years of age) with CP were included in the study and randomized into the TFBM (GFBM [n = 5]) and placebo (RG [n = 3]) groups. The intervention consisted of 12 sessions, twice a week, with application of the TFBM in the GFBM in seven regions of each lower limb (cluster 850 nm, 3,276 J, 3 J/cm²) and simulation of application in the SG. The Polar device (RS800CX) was used to record the R-R intervals and the FLIR E8 WI-FI (FLIR® Systems, Inc.) thermal imaging camera, with a resolution of 320 x 240 pixels, was used for the TIV. Statistical analysis was performed using the Bonferroni post-hoc repeated measures ANOVA test,

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and the significance level adopted was $p < 0.05$. Results: A significant difference was observed in post-intervention status for iRR ($p = 0.035$), pNN50 ($p = 0.047$), and heart rate ($p = 0.018$). The iRR and pNN50 data are parasympathetic markers and showed increased values for post-time in the GFBM. For heart rate, an indicator of sympathetic behavior, there was a decrease in GFBM. Although the IVT did not present a statistically significant difference, there was an increase in facial temperature and a decrease in peripheral temperature in the GFBM, suggesting a reduction in the stress condition. Conclusion: The TFBM showed promising results of greater influence of the parasympathetic nervous system and modulation of the sympathetic nervous system, which can promote better health conditions.

Keywords: International Classification of Functionality. Disability and Health. Cerebral palsy. Autonomic Nervous System. Sympathetic Nervous System. Autonomic variables.

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INTRODUCTION

Cerebral palsy (CP) is characterized by a non-progressive group of motor disorders caused by injury to the immature brain. Approximately 92% of CP cases are attributed to the perinatal period, even in preterm births (Vitrikas; Dalton; Breish, 2020). Although not progressive, CP is often mutable, according to the functional limitations that arise during development. This fact leads to some questions, as described by Rosenbaum (2022), *"Should all changes be attributed to motor disabilities? Is it possible to promote lifelong health strategies, from an early age, that result in healthier and more sustainable functional lives for people with CP?"*

In order to seek new answers for the development of individuals with CP, studies are being carried out on the behavior of the autonomic nervous system (ANS), responsible for regulating body homeostasis, adapting biological responses according to environmental variations (Dan, 2017). ANS imbalances trigger a cascade of health-related complications and may be related to the occurrence of morbidities and increased risk of mortality (Katz-Leurer; Amichai, 2019). Knowing the autonomic behavior of individuals with CP could lead to an understanding of different stimuli and their respective responses, a condition observable by parameters such as heart rate (HR), body temperature, and muscle contraction (Amichai; Katz-Leurer, 2014), motivating the planning of different prevention and rehabilitation strategies.

One parameter of autonomic evaluation is heart rate variability (HRV), which presents lower values during rest and exercise in children with CP compared to those with typical development (Kholod; Jamil; Katz-Leurer, 2013), as well as in stressful situations (postural changes or head position) (Amichai, 2019). These observations indicate a deficit in the ability to adapt to the environment due to the inadequate physiological functioning of this population (Vanderlei et al., 2009).

Another way to observe the functioning of the ANS is through the surface temperature of the skin, as it is regulated by sweating and subcutaneous vascular activity, controlled by the ANS (Uddin et al., 2023). Infrared thermography (IVT) is a rapid, remote, non-invasive, and replicable way that monitors body temperature (Lahiri et al., 2012). IVT has been widely used in medicine in the diagnosis and detection of health problems, such as breast cancer, peripheral vascular disorders, and heart and kidney diseases (Lahiri et al., 2012). In addition, the IVT is able to pick up thermal changes associated with moments of stress (Vinkers et al., 2013).

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Photobiomodulation therapy (TFBM) or low-intensity light therapy is a non-invasive and low-cost therapy that, through light energy, generates biochemical, bioelectrical, bioenergetic, and biostimulatory effects (Lin et al., 2020). Depending on the wavelength and dose used in different tissues, BMFT has diverse responses, such as increased adenosine triphosphate synthesis, blood flow and brain energy metabolism, stimulation of angiogenesis, antioxidant actions, neurobehavioral and emotional responses, and improvement of sleep disorders (Hamblin et al., 2019; Chang et al., 2022), in addition to neuroprotective effects and modulation of immune cells and inflammatory cytokines (Hennessy; Hamblin, 2017), generated both by local application and by systemic repercussions and distant from the focus of irradiation (Yang et al., 2018).

TFBM has shown promising and positive effects in different populations, such as individuals with fibromyalgia, who report improved sleep and tiredness, indicators of good ANS functioning (García et al., 2011). However, its effects are not yet fully understood and, to date, no studies have been found on the possible effects of BMT-MTT on the ANS of children and adolescents with CP.

Thus, the hypothesis of this study was that BMT-MTR applied to large muscle groups of children and adolescents with CP would produce systemic effects on ANS regulation, as evidenced by the analysis of HRV and IVT.

OBJECTIVE

To verify the effect of BMT-MBT on autonomic variables in children and adolescents with CP.

MATERIALS AND METHODS

This is a randomized controlled clinical trial, submitted to and approved by the local Research Ethics Committee, registered on the Brazilian Clinical Trial Registry (ReBEC) platform and carried out at the university's clinic. All participants and guardians were informed about the study procedures and signed the free and informed consent form and, when possible, the participant signed the free and informed consent form, as determined by Resolution 466/12 of the National Health Council.

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PARTICIPANTS

The study had 12 participants, aged between 7 and 13 years, male and female. The inclusion criteria for the study were to have a diagnosis of CP, to be classified between levels I to V of the Gross Motor Function Classification System (GMFCS), to be female or male, and to be aged between 6 and 14 years. Individuals with neoplastic foci, hemorrhage episodes, thermal sensitivity disorders, active infections, or who were using photosensitive drugs were not included. In addition, two participants were excluded during the reassessment due to flu-like conditions, which interfered with data collection.

The sample was selected through a survey of patients treated at the CER/CEES and at the Amor de Criança Project, linked to the Beneficent Hospital of the University of Marília (UNIMAR). The proposal to participate in the project was made by telephone or in person, with guidelines for an initial evaluation, if they were interested in participating.

The sample was by convenience (n = 12) and the participants were randomized by simple draw into two groups: the photobiomodulation group (GFBM), submitted to BMT, and the placebo group (GP), in which the BMT-BMT simulation was performed. The intervention protocol (effective application and simulation) was carried out over 12 sessions, twice a week, and up to three absences were allowed, which, if they occurred, would be rescheduled, ensuring the completion of the total number of sessions proposed. If the number of absences allowed was exceeded, the applications would be discontinued.

EVALUATION PROTOCOL

The evaluations were carried out at two different times: before the intervention and immediately after the conclusion of the 12 sessions, being conducted in a room with controlled temperature between 21°C and 24°C.

To collect HRV data, the participant remained in a comfortable posture, in the supine position, on the platform covered with a thick cotton fabric, for approximately one hour. For the purpose of standardizing data collection, the period of the first evaluation of the participants was maintained for reevaluation.

Heart rate variability

HRV was assessed using a Polar RS800CX cardiofrequency meter (Polar Electro, Finland) attached to an appropriate strap and positioned on the chest, in the region of the distal third of the sternum. The heart rate receiver remained in the evaluator's hand. The duration of the recording of the heart records was 20 minutes, where the participant had to remain lying at rest on the platform, after familiarizing themselves with the environment.

For the analysis of HRV indices, heart rate was recorded beat-by-beat throughout the experimental protocol with a sampling rate of 1000 Hz. Stable series with 256 RR intervals were selected (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). In these series, digital and manual filtration were performed to eliminate premature ectopic beats and artifacts, and only those with more than 95% of sinus beats were included in the study (Vanderlei et al., 2009). For the analysis of linear indices in the frequency and time domains, the Kubios HRV 2.1 analysis® software (Niskanen et al., 2004) was used.

The analysis of HRV indices can be performed using linear methods (in the time and frequency domain) and nonlinear methods. In the present study, the means of the variables in the time domain were used, which included: mean value of the R-R intervals (iRR [ms]), standard deviation of all RR intervals (SDNN [ms]), square root of the mean of the sum of squares of the differences between adjacent RR intervals (RMSSD [ms]), percentage of adjacent RR intervals that would differ more than 50 ms in duration (pNN50 [%]). Regarding the nonlinear methods, the standard deviation of the instantaneous variability of the RR interval (SD1) was used (Vanderlei et al., 2009).

Infrared thermography

In the TIV analysis, the thermal radiation emitted by the skin surface was detected by an infrared camera and the intensity of the emitted radiation was converted into temperature on the Celsius scale (Lahiri et al., 2012).

The FLIR E8 WI-FI thermal imaging camera (FLIR® Systems, Inc.) was used, with a resolution of 320x240 pixels, sensors that allowed the evaluation of temperatures in the ranges of -20°C to +250°C, sensitivity <0.06 °C and accuracy of ±2 °C, according to information from the manufacturer. The camera was positioned and directed to the face and lower limb (LL) region. Mean temperature data were analyzed across different body regions.

To mean the temperature in the face, the triangle of the face was used, considering the outer corner of each eye and the mental protuberance (Figure 1), and to mean the temperature in lower limbs, the middle third of the thigh (Figure 2), forming a rectangle in each limb and calculating the mean between them.

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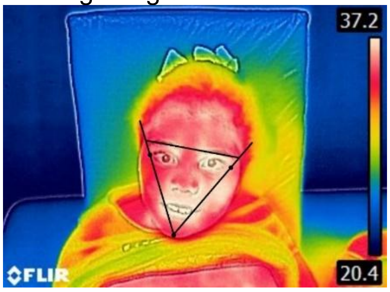
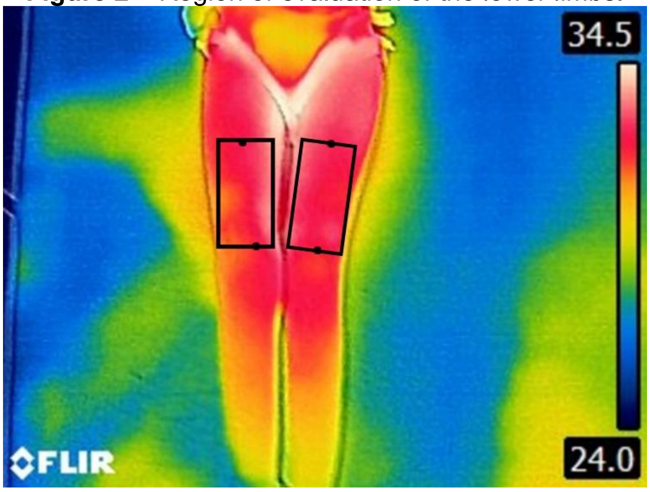
Figure 1 – Triangle region of the face for evaluation.



Source: personal archive, 2024.

Figure 2 – Region of evaluation of the lower limbs.



Source: personal archive, 2024.

Intervention protocol – Photobiomodulation therapy

For the TFBM protocol, the G1 Antares® cluster model (IBRAMED, Amparo, SP, Brazil) was used, containing 13 diodes with a wavelength of 850 nm and an output power of 500 mW each, totaling 6,500 mW, with continuous mode emission and a contact area of 80 cm2. The energy used was 234 J (3 J/cm2) per point of application (seven in each lower limb), totaling 3,276 J when considering 14 points.

The cluster was positioned in contact with the participant's skin, at an angle of 90°, in order to minimize the loss of energy emission at each application point. When the cluster's contact area was larger than the participant's lower limb, the limbs were positioned side by side, considering this configuration as a single point. The application time per point was 36 seconds, totaling 504 seconds (or 8.4 minutes) for the total application in the 14 points. Between applications in the same region, a minimum interval of one minute was respected.

The GFBM participants underwent 12 sessions of TFBM, twice a week with a one-day interval between them, and the participants of the GP underwent the same number of simulations of the TFBM. The simulations were performed with the same procedures as the actual applications: programming the device with the correct parameters, use of protective goggles by the applicator and participant, and coupling of the cluster head at the intervention points; with the only exception of not pressing the button that would start emitting light. To control the time, a stopwatch was used by the applicators in order to count 36 seconds for each point of application.

STATISTICAL ANALYSIS

After verifying the normality and homogeneity of the data, the Bonferroni post-hoc repeated measures ANOVA test was used to verify intra- and intergroup variables. The level of significance was $p < 0.05$.

RESULTS

A total of 12 participants were included in the study, who were evaluated and randomized into two groups: GFBM (n = 8) and SG (n = 4). All individuals completed the evaluation protocol and completed the 12 intervention sessions. However, two children from the GFBM had flu-like symptoms during the reassessment, which made it impossible to use their data. In addition, two other children, one from the GFBM and the other from the SG, were excluded from the analysis due to failures in the recording of data by the cardiofrequency meter.

The sample analyzed (n = 8), with a mean age of 8.75 ± 1.67 years, was divided between GFBM (n = 5) and SG (n = 3), with four (80%) and two girls (66.7%) in each, respectively. Regarding the GMFCS, the GFBM had four individuals classified as GMFCS V (80%) and one as GMFCS III (20%), while the GP was composed entirely of individuals with GMFCS V (100%). Table 1 presents the characterization of the sample, with no significant difference between the data.

Table 1 – Characterization of the sample with mean and standard deviation values

Features	GFBM (n=5)		GP (n=3)		ANOVA (p)
	Average	DP	Average	DP	
Age (years)	8,4	1,95	9,33	1,15	0,487
Mass (kg)	28,14	12,24	34,76	16,38	0,387
Height (m)	1,2775	0,12	1,22	0,14	0,534
BMI (kg/m²)	15,86	4,58	22,63	10,77	0,248

Legend: GFBM: photobiomodulation group; PG: placebo group; SD: standard deviation; kg: kilograms; m: meters; BMI: body mass index; ANOVA: One Way analysis of variance.

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Tables 2 and 3 show the comparative analysis by the ANOVA test of repeated measures with Bonferroni's post-hoc test, considering a significance level of $p < 0.05$. When analyzing the results in the repeated measures ANOVA test, it was observed that results related to the moment were significant or very close to presenting significance, and for this reason it was decided to also analyze the Bonferroni post-hoc test.

Table 2 – Heart rate variability indices at the time points before and immediately after the last intervention.

		GFBM (n=5)		GP (n=3)		ANOVA (p)		
		Average	DP	Average	DP	A moment	Group	Interaction
iRR (ms)	Pre	547,84	103,69	510,63	96,71	**0,035***	0,535	0,917
	Post	675,84	127,11	628,73	79,04			
	% Δ	23,36		23,13				
	p	0,061		0,151				
RMSSD (ms)	Pre	19,56	10,66	14,27	11,07	0,057	0,467	0,679
	Post	48,30	34,04	34,03	17,80			
	% Δ	146,93		138,55				
	p	0,064		0,272				
pNN50 (%)	Pre	3,76	3,71	3,13	5,08	**0.047***	0,554	0,583
	Post	26.28 ‡	22,66	17,13	14,44			
	% Δ	598,94		446,81				
	p	**0,046**		0,273				
SD1 (ms)	Pre	13,86	7,56	10,13	7,86	0,058	0,466	0,677
	Post	34,24	24,09	24,10	12,59			
	% Δ	147,04		137,82				
	p	0,064		0,273				
SDN (ms)	Pre	47,86	44,37	42,13	25,48	0,677	0,614	0,595
	Post	59,44	35,20	40,70	19,75			
	% Δ	24,20		-3,40				
	p	0,446		0,940				
HR (bpm)	Pre	113,10	19,51	121,62	23,44	**0,018***	0,593	0,8
	Post	92,16	18,01	96,92	12,73			
	% Δ	-18,51		-20,31				
	p	0,053		0,07				

Legend: GFBM: photobiomodulation group; PG: placebo group; SD: standard deviation; iRR: R-R intervals; RMSSD: square root of the mean of the square of the differences between adjacent normal RR intervals over a time interval; SD1: dispersion of points perpendicular to the line of identity; SDNN: standard deviation of the R-R intervals; HR: heart *rate*; ms: milliseconds; bpm: beats per minute; *: statistically significant difference for the time in the repeated measures ANOVA test; ‡: statistically significant difference in relation to the pre-intragroup moment; *: significance level of p<0.05; % Δ: percentage of variation between before and after the last intervention.

The IVT results did not show statistically significant differences. However, as in the analysis of HRV, the result related to the interaction in the face was significant in the GFBM, so it was decided to also analyze the Bonferroni post-hoc test.

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Table 3 – Infrared thermography data at the moments before and immediately after the last intervention.

		GFBM (n=5)		GP (n=3)		ANOVA		
		Average	**DP**	**Average**	**DP**	**A moment**	**Group**	**Interaction**
FACE	Pre	34	1,76	35,26	0,91	0,082	0,736	**0,026***
	Post	34,9	1,25	34,26	0,06			
	p	0,064		0,099				
	% Δ	2,65		-2,84				
MMII	Pre	32,43	0,72	32,26	1,61	0,740	0,481	0,448
	Post	32,67	1,47	31,66	1,36			
	p	0,845		0,376				
	% Δ	0,74		-1,86				

Legend: GFBM: photobiomodulation group; PG: placebo group; SD: standard deviation; LLLL: lower limbs; ANOVA: One Way analysis of variance; % Δ: percentage of variation between before and after the last intervention.; *: statistically significant difference, p<0.05.

DISCUSSION

HEART RATE VARIABILITY (HRV)

The present study was conducted with the aim of verifying whether BMT-MTT would have effects on the autonomic adjustment of children and adolescents with CP, since autonomic instability is a common condition after brain injury and can be manifested by the continuous activation of sympathetic pathways (Kholod; Jamil; Katz-Leurer, 2013).

The HRV results of this study, presented in Table 2, showed statistically significant differences after 12 sessions of BMT, in the condition at the time of the ANOVA test, for iRR, pNN50 and HR. Both groups showed an increase in the mean values of iRR, RMSSD, pNN50 and SD1 (indicators of parasympathetic activity) at the post-application moment, possibly due to the familiarity of the participants with the researchers and with the environment, reducing the tension generated by the unknown. However, the behavior of the data in the intragroup analysis showed a significant difference for pNN50 in GFBM and a significant non-significant increase in iRR, RMSSD, and SD1 also in the GFBM group, suggesting an increase in parasympathetic activity, as an effect of TFBM (Larsson et al., 2023).

Although sympathetic activity is important for the protection of the body under conditions of stress, the increase in its activity, for long periods, accompanies a decreased parasympathetic function, generates imbalances for an inflammatory response and dysfunction of the intestinal microbiota, associated with a cascade of events harmful to body homeostasis (Bellocchi et al., 2022), reinforcing the need to develop strategies for the modulation of parasympathetic activity in the population of individuals with CP.

In the GFBM, the values of the percentage of variation of the SDNN increased by 24.19%, suggesting a positive effect on the decrease in the anxious state after the use of TFBM (Hamblin et al., 2019; Chang et al., 2022, Montazeri et al., 2022), different from the behavior of the GP, which had a decrease of 3.39%. In HRV analyses, the SDNN was shown to represent global ANS activity and its decreased values were associated with a worse anxiety condition (Larsson et al., 2023), as well as the pNN50, used to reflect vagal or parasympathetic tone (Pham et al.2021).

Higher HR values signal increased sympathetic activity behavior (Larsson et al., 2023), related to disease conditions (Vanderlei et al., 2009) and possible decrease in body homeostasis status (Gasior et al., 2020).

In the present study, HR values in the GFBM showed a more pronounced decrease, with values close to statistical significance only in GFBM by Bonferroni's post-hoc test, which suggests a decrease in sympathetic activation and better cardiac autonomic control. The increased activation of sympathetic pathways associated with low vagal tone shows negative outcomes related to mood disorders and increased risk of developing comorbidities (Pham et al.; 2021).

Thus, the TFBM showed a tendency to increase vagal tone and decrease sympathetic tone, which could be used as a resource to modulate general health conditions and as a regulator for psychological issues.

INFRARED THERMOGRAPHY (TIV)

The ANS regulates body temperature through sweat production and vasomotor activity of the subcutaneous vessels (Gioia et al., 2023). Changes in blood flow, such as increase or decrease, occur due to situations of greater or lesser activation of the sympathetic ANS (Nakayama et al. 2005). In this way, body temperature can be considered an index of sympathetic activity and a predictor of stress.

The literature indicates that, in situations of stress, peripheral temperature tends to decrease, due to physiological mechanisms involving the fight or flight response, as demonstrated by Vianna et al. (2005), who observed a reduction in the temperature of the tail and paws in rats conditioned to fear. In the present study, there was a small increase in temperature in the lower limbs in the GFBM, while the opposite occurred in the SG, which suggests a less prevalent sympathetic activity post-intervention, with a reduced stress state.

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The temperature of the face showed an increase in GFBM, while the opposite was observed in the SG. Previous studies have pointed out that the increase in axis temperature after stressful situations indicates greater sympathetic activity (Uddin et al., 2023; Ludwig, et al., 2007; Vianna et al.; 2005). However, Merla et al. (2007) demonstrated that in the condition of pain or fear of feeling pain, there was a general reduction in facial temperature, due to sudomotor activity and peripheral vasoconstriction.

The evaluation of thermographic images of the face can be performed in different regions, each area representing a greater or lesser relationship with sympathetic activity. For example, regions such as the forehead, cheeks, and medial part of the eyes (or lacrimal caruncle) have been linked to greater sympathetic activity when with higher temperatures (Vinkers et al., 2013; Stewart et al., 2007; Uddin et al., 2023). While the nose, an extremity, tends to have a reduction in temperature soon after stressful situations (Gioia et al., 2023).

Another possible explanation for the increase in facial temperature in GFBM is that, in the analysis performed, an area of the face was used in which the nose was the only region without cuts. This implies that its area was larger relative to the other regions, such as the forehead and cheeks, which generally have larger areas (Vinkers et al., 2013). The increase in nose temperature, possibly caused by stress reduction, may have been more relevant in comparison with the other regions, influencing the increase in the mean value of facial temperature, which indicates a possible reduction in sympathetic activity in GFBM.

It is important to highlight that, to date, there are still few studies on the measurement of temperature by means of IVT in situations of chronic stress, especially in populations that present a constant sympathetic imbalance, such as the one in this study. In the literature found, the stress conditions were acute, such as in animals conditioned to fear or evaluations of stressful situations and tests in humans, as described by Vianna et al. (2005) and Vinkers et al. (2013), for example.

The association between IVT and HRV data demonstrated an attenuation of the sympathetic system and prevalence of parasympathetic activity, which translates into reduced stress and greater autonomic control in GFBM.

HRV can be considered a more appropriate autonomic marker than IVT (Engert et al., 2014). However, thermal imaging has the advantage of being remote, non-invasive, and the absence of the need for prolonged rest for data collection, which makes it advantageous in the evaluation of children and adolescents with CP.

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STUDY LIMITATIONS

As this is a small number of participants, the data obtained should be interpreted as preliminary. The sample size may have interfered with HRV data, which are sensitive to measurement and require rest for a reliable measurement. During the recording, some participants remained agitated and made sudden movements. This difficulty may have influenced data collection, even though the areas of more stable behavior were carefully selected.

The difficulty in assessing HRV in this population highlights the need for further studies aimed at standardizing this measure, in order to facilitate its application both in clinical practice and in scientific research.

CONCLUSION

The results of the TFBM pointed to promising information in the activation of the parasympathetic nervous system and modulation of the sympathetic nervous system, which may promote better health conditions for individuals with CP.

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