

STUDY PROTOCOL

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Action observation therapy effects on motor function and balance in cerebral palsy: an fNIRS-based randomized trial

Melike Özipek^{1*} , Mevhibe Saricaoglu^{2,3}, Lütfü Hanoğlu^{3,4} and Fatma Mutluay⁵

Abstract

Cerebral palsy (CP) is an umbrella term for movement, motor, and posture disorders that occur in the developing brain from any non-progressive cause. Action observation therapy (AOT) is a method used in the treatment of motor disorders. Here, the manuscript presents the study protocol for the effect of AOT on balance using functional near-infrared spectroscopy (fNIRS). This study protocol aims to comprehensively evaluate the impact of AOT in patients with CP regarding brain activation and behavioral and motor symptoms. This is a single-center randomized controlled trial for patients with CP. Patients determined eligible according to the study criteria will be randomized into two treatment groups: the action observation therapy group and the control group. Each group will include 12 patients, and the age range is 5–15. The patients will be treated for 15 sessions for three consecutive weeks. In this study, AOT will be applied to improve balance. The following tests will be applied to evaluate the patients' motor function and balance skills before rehabilitation. Gross Motor Function Measure-88 (GMFM-88)-D and E parts, Lower Extremity Selective Control Evaluation Scale (SCALE), The Trunk Impairment Scale (TIS), Pediatric Balance Scale, Nintendo Wii-Fit Balance Score, Timed Up and Go Test (TUG), and Pediatric Data Collection Tool (PODCI) will be applied. Brain regional blood flow changes during static and dynamic balance will be evaluated with fNIRS. All evaluations will be performed before treatment and repeated after treatment.

Trial registrations Study was approved by the Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (Ethical Report No: E-10840098-772.02-3957), registered at ClinicalTrials.gov with the number NCT05995197, 08.09.2023.

Keywords Action observation therapy, Cerebral palsy, Balance, fNIRS

Significance statement

In this study, it is hypothesized that AOT will positively affect both brain activation and motor symptoms by enhancing static and dynamic balance functions in individuals with cerebral palsy. We hope that the contributions of AOT to CP rehabilitation will be unveiled, leading to increased participation among children in the treatment. In line with the anticipated data, fNIRS is expected to emerge as an important technique for demonstrating the treatment's effects and proving its effectiveness.

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Introduction

Cerebral palsy (CP) is an umbrella term for movement, motor, and posture disorders that occur in the developing brain from any non-progressive cause [1].

Along with the motor disorders of CP, sensory, perception, cognition, communication, and behavioral disorders, epilepsy, and secondary musculoskeletal system problems are often observed [2]. Muscle weakness in the skeletal muscles, pathological reflexes, and simultaneous contraction of agonist and antagonist muscles can lead to balance problems due to delayed responses of the ankle muscles and loss of postural control [3]. Balance impairment in CP causes limitations in upper extremity functions, daily living activities, social roles, and participation in society [4].

The management of CP, a condition that affects multiple aspects of a child's development, typically incorporates motor, sensory, and cognitive strategies. These interventions can be used independently or synergistically to maximize functional outcomes. In recent years, cognitive strategies such as action observation therapy (AOT) have begun to be combined into all rehabilitation practices [5–7]. Action observation is based on the individual observing actions performed by others without any motor practice. This technique stimulates motor areas in the brain, and mirror neurons in the occipital, temporal, parietal areas, inferior parietal lobule, and inferior frontal gyrus are activated [8]. It is stated that action observation is an effective method for learning or developing motor skills [7, 9]. Although it has been used in many diseases recently, it is especially preferred in treating motor disorders of patients with CP [10–14].

In CP, balance impairments are primarily attributed to deficits in selective motor control, the persistence of primitive reflexes, and inadequate postural adjustments [15]. These motor dysfunctions hinder the development of stable and coordinated movement patterns necessary for balance. AOT has emerged as a promising alternative to traditional rehabilitation approaches. It is based on the principle that observing goal-directed actions activates the mirror neuron system, thereby facilitating motor learning and neuroplastic changes in the motor cortex [16]. By combining action observation with subsequent physical execution, AOT enhances motor planning and memory formation, which can ultimately improve postural control and balance in children with CP [3].

In recent years, functional near-infrared spectroscopy (fNIRS) stands out as a noninvasive, reliable, easy-to-use, low-cost method. fNIRS records hemodynamic changes in brain oxygenation due to increased cerebral blood flow in the activated cortical area [17]. It differs from other methods in that it is tolerant to body movements and less sensitive to motor movement noises; the device does

not have a restricted environment, is portable, and allows simultaneous measurement while the person is performing a motor task or is in motion [18]. fNIRS is used to investigate brain activation during many applications involving motor tasks or rehabilitation processes, such as sitting and standing, walking, running, and stepping [19].

Studies have shown that fNIRS is used to reveal the disease's pathology and show the rehabilitation effect in patients with CP [14, 20–22]. On the other hand, it has been reported that AOT contributes to the healing of different diseases as an effective treatment method [3, 6–8, 23, 24]. In summary, previous studies regarding AOT focus on upper extremity functional recovery and rehabilitation in children with CP. However, action observation is thought to be practical in all motor skills by facilitating motor learning in the learning process [23]. Mere observation of actions is part of the complex mechanism of motor learning: it is stated that action observation can evoke an internal representation of the observed movement in the brain, strengthening the formation of motor memory and facilitating subsequent motor performance [6]. The improvement in motor functions after AOT is reported to be associated with increased cortical activation in the bilateral ventral premotor area, inferior parietal area, supplementary motor area, contralateral supramarginal gyrus, and bilateral superior temporal gyrus [24]. However, the studies conducted are insufficient to investigate AOT, mainly gross motor functions and balance, and the necessity of future studies to be evaluated in combination with neuroimaging techniques comes to the fore, considering the relationship of AOT with cortical activation.

This study aims to investigate the effectiveness of AOT in children with CP and reveal the effect of these approaches on brain activation with the fNIRS technique. In particular, this protocol sample is an essential step in filling the gap in the literature and improving the motor and balance functions of individuals with CP.

Our research hypothesizes that AOT in individuals with CP will positively affect both brain activation and motor symptoms by increasing static and dynamic balance functions. This effect will be evident when compared to the control group. This study provides a comprehensive approach using various motor tests in conjunction with the fNIRS technique to evaluate the effect of AOT on balance in CP patients.

Methods

The study has four stages. The first stage is the selection of patients and their inclusion in the study; the second stage is the pre-rehabilitation evaluation; the third stage is the rehabilitation process; and finally, the fourth stage is repeating the evaluations to show the post-rehabilitation

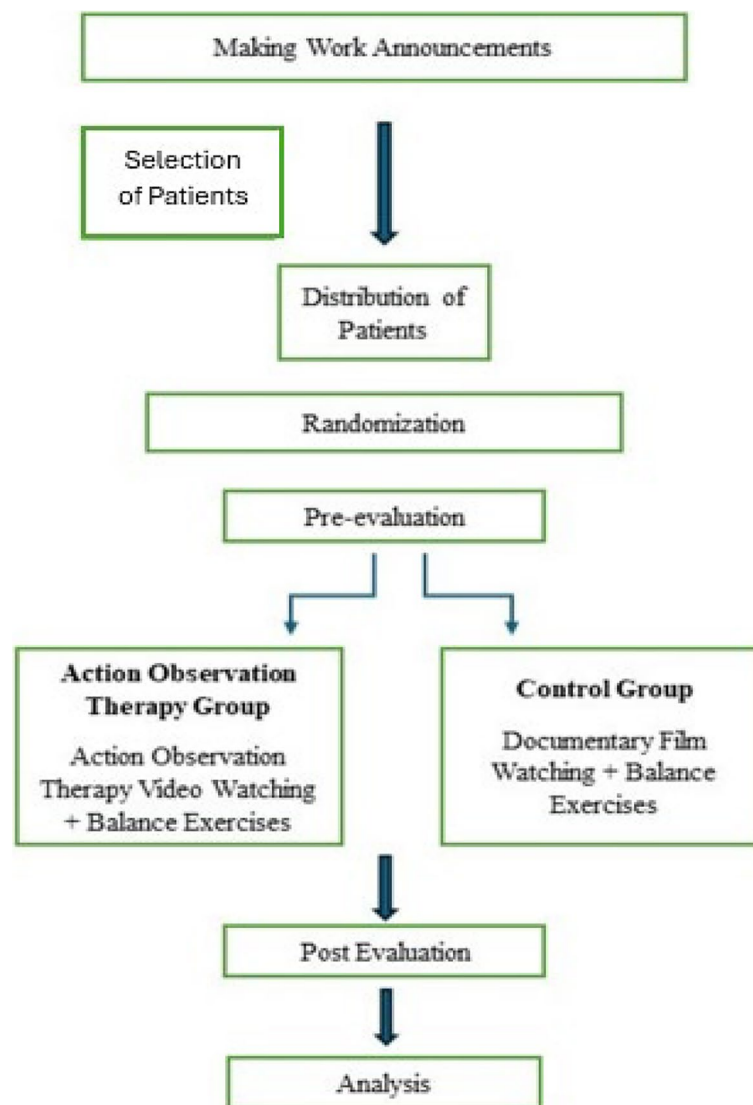


Fig. 1 Flow diagram

effect (Figs. 1 and 2). The study was designed according to the guidelines of the Declaration of Helsinki. It was approved by the Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (Ethical Report No: E-10840098-772.02.02.02–3957), registered at ClinicalTrials.gov with the number NCT05995197. The relevant rules and regulations will carry out all methods, and written informed consent will be obtained from the patients and their relatives.

Selection of patients and inclusion in the study

The patient population is children aged 7–15, diagnosed with CP, of both genders, who meet the inclusion criteria and volunteer to participate in the study. The G*Power

program was used to determine if there was a sufficient sample size for the study.

In line with the fNIRS task design, planned contrasts with $f=0.70$ within and between repeated measures will have the power of 0.90 at an alpha error probability of 0.05 to detect 24 patients (Cohen 1988). Because no prior study can provide direct information about the expected effect size for different outcome measures, this number is similar to the number of patients and effect strength in both fNIRS studies and studies demonstrating the action observation effect.

To reach the patients, e-mails will be sent to the health institutions, and face-to-face and telephone interviews will be held with the institutions that respond positively.

	Enrolment	Allocation	Assessments and Treatment Programme					Close-out
TIMEPOINT**	-t ₁	0	t ₁	t ₂	t ₃	t ₄	t ₅	t _x
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Allocation(randomization)		X						
INTERVENTIONS:								
[Action Observation Therapy Group]			•	—————				
[Control Group]				—————				
ASSESSMENTS:								
GMFM-88 D and E Parts, SCALE, TIS, Pediatric Balance Scale, Nintendo Wii-Fit Balance Score, TUG, PODCI Scales, fNIRS			X				X	
			X				X	X

Fig. 2 SPIRIT figure

The study will be explained in detail. Criteria for inclusion in the survey: being diagnosed with CP, being between the ages of 7–15, being at level I and II according to the Gross Motor. According to the Health Board report, only motor skills are affected. Patients are classified using the Function Classification System, have spastic type cerebral palsy, cooperate with evaluations, and do not have vision or hearing loss. Exclusion criteria from the study were having received botulinum toxin application or a surgical operation within the last 6 months and having a diagnosis of dyskinetic, ataxic, and hypotonic type CP. Patients and their relatives who meet the inclusion criteria will be invited to be evaluated and begin the study.

Before rehabilitation, all patients' history and demographic information will be taken, and pre-and post-treatment evaluation measurements will be made by a physiotherapist who is competent in pediatric evaluation. Gross Motor Function Measure-88 [25] (GMFM-88)-D and E parts, Lower Extremity Selective Control Evaluation Scale (SCALE) [26], Trunk Impairment Scale (TIS) [27], Pediatric Balance Scale [28], Nintendo Wii-Fit Balance Score [29], Timed Up and Go Test (TUG) [30], and Pediatric Data Collection Tool (PODCI) [31] will be

applied. They will be asked to report whether they have previous experience with fNIRS and other neuroimaging techniques.

Experimental set-up and equipment

fNIRS recordings will be obtained from the fNIRS device (Medical Technologies LLC, Berlin, Germany) using the NIRStar Acquisition (NIRx Medizintechnik GmbH, Germany) program. Recordings from 20 channels of 8 sources, 8 detectors, and 34 optodes will be taken. Optodes will be positioned on the premotor cortex, supplementary motor area, and primary motor cortex to scan motor areas by the EEG 10–20 system (Fig. 3). The distance between optodes will be adjusted to a maximum of 3.4 cm. Sampling rate is 3.47 Hz. Recordings will be made with wavelengths of 760 nm and 850 nm [32]. The appropriate NIRS cap will be selected to ensure a proper fit on patients' heads. The recording will be taken in a dark environment. As the final step before recording begins, calibration will be performed to determine the appropriate amplification factor for each source-detector combination and evaluate signal quality. The record will be launched if every channel in the signal quality map

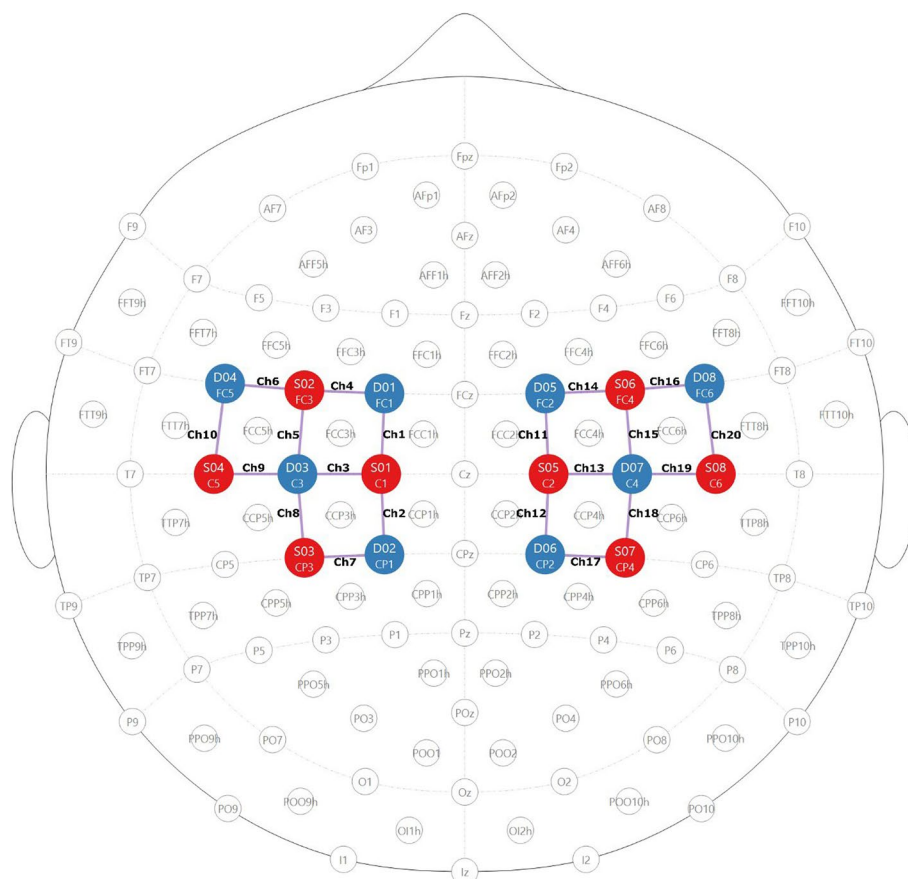


Fig. 3 fNIRS recording assembly (red represents source, blue represents detector, lines represent channels; S: source; D: detector; Ch: channel)

(generated by the NIRStar software) is labeled as “excellent” or at least “acceptable.”

Pre-rehabilitation assessment and fNIRS recordings

The following tests will be applied to evaluate the patients’ motor function and balance skills before rehabilitation:

Gross Motor Function Classification System: The Gross Motor Function Classification System (GMFCS) for CP is based on the child’s self-initiated movements, emphasizing sitting, shifting, and mobility. The main criterion when defining the five-level classification system is that the differences between the levels are meaningful in daily life.

- Level 1: Walks without restrictions.
- Level 2: Walks with restrictions.
- Level 3: Walks using hand-held mobility devices.
- Level 4: Self-mobility is limited. The person can use a motorized mobility device.
- Level 5: Transported in a manually pushed wheelchair.

Gross Motor Function Measure-88: Gross Motor Function Scale (GMFM-88) is divided into five main sections. It consists of 88 items, 17 of which are in the supine-prone position and turning section, 20 in the sitting section, 14 in the crawling-kneeling section, 13 in the standing section, and 24 in the walking-running-stair climbing section. It is scored between 0 and 3 depending on how many activities are done. If the activity cannot be started, 0 is given; if it is started independently, 1; partially completed, 2; and if it is completed independently, 3 points are given. Performance is calculated as a percentage of the scores obtained from all sections. An analysis determines whether the expected figure can be reached according to the child’s age and current gross motor movement capacity (GMFCS) [25].

Selective Control Assessment of the Lower Extremity (SCALE): SCALE is currently used to evaluate selective motor control of the lower extremity. The hip joint is evaluated separately, with the hip joint in the side-lying position and the knee, ankle, subtalar joint, and toe joints in the sitting position. The patient is asked to perform the desired movement for each joint level in approximately 3

s and with a specific verbal tempo. The degree of selective motor control is determined for each joint as “normal” (2 points), “impaired” (1 point), or “unable” (0 points) according to the patient’s ability and shape to perform movements. The total score is obtained by summing up the scores obtained from the joint levels for each extremity to 10 points. A high score means that selective control is reasonable [26].

Trunk Impairment Scale (TIS): The TIS functionally evaluates the trunk in terms of strength in the sitting position. It consists of three subsections: static, dynamic, and coordination. The highest scores that can be obtained from the static, dynamic, and coordination subheadings are 7, 10, and 6 points. The total TIS score varies between 0 and 23. A high score indicates that the static and dynamic balances of the trunk and trunk coordination are good [27].

Pediatric Data Collection Tool (PODCI): PODCI is one of the scales with proven validity and reliability, prepared for children and adolescents and frequently used to determine functional status and evaluate health-related quality of life. The scale has three different forms:

- a) PODCI child form: The section consisting of 86 questions is filled out by the families of children between the ages of 6–10.
- b) PODCI adolescent family form: The section consisting of 86 questions is filled out by the families of adolescents between the ages of 11–18.
- c) PODCI adolescent form: The section consisting of 83 questions is filled out by adolescents between the ages of 11–18.

As a result, the subscale standardized score of “0” indicates that the health status and quality of life are worse, and the score of “100” indicates that they are at a good level [31].

Pediatric Balance Scale: The Pediatric Balance Scale is a modified version of the Berg Balance Scale used to evaluate school-age children’s functional balance skills. The scale consists of 14 items scored from 0 points (lowest function) to 4 points (highest function) with a maximum score of 56. A high score means that the balance function is reasonable [28].

Nintendo Wii-Fit Balance Score: After the patient’s demographic information is recorded in the device system, the patient is shown how to place his foot on the Nintendo Wii-Fit balance board; he is asked to stand on the balance board without moving, and the ratio of weight given to the right and left feet (body center of gravity on the right and left foot) is evaluated, and the data is recorded as a percentage. Although the Nintendo Wii-Fit Balance device is used for different purposes and

games, it will be used in the study to evaluate the weight transfer rate on both feet [29].

Timed Up and Go Test (TUG): It is a valid and reliable method for evaluating functional mobility and static and dynamic balance in CP. From a starting position with hips, knees, and ankles flexed at 90°, patients rise from a chair without arm support, walk 3 m, return, and sit in the chair. The duration begins to be recorded with the “go” stimulus given by the evaluator and is stopped when the hip touches the chair. This process is repeated three times, and the therapist records the performance in seconds. A short duration indicates good balance performance [30].

fNIRS recording assessment

The study aims to familiarize each patient with the tasks before starting fNIRS recordings. During this familiarization period, subjects will be asked to watch two videos on balance tasks: standing on hard and soft ground. Both balance task videos show a person standing normally with an upright posture without any movement.

An essential point in evaluating the balance function is the ground used. The ground used in the balance test varies depending on the purpose and the person’s balance level, and hard and soft ground are most often preferred. Hard ground provides a more stable surface and makes it easier for the person to maintain balance. Soft ground, on the other hand, is a more variable and unstable surface, thus making it difficult for the person to maintain balance. Regarding proprioception, muscle activation, and coordination, soft ground appears to have more sensitive properties for balance assessment. Neuroimaging studies show that the activity in motor centers is higher in more challenging dynamic tasks than in static standing, and balance assessment on soft ground is expected to be higher than on hard ground [33]. Previous fNIRS studies investigating standing balance have shown a continuous increase in prefrontal cortex activity with increasing balance difficulty [34]. Based on all this information, the experimental task was performed on both a hard surface and a soft surface to demonstrate the effect of AOT.

fNIRS recordings will then be performed with a 3-min break between each experiment. During fNIRS recordings, experiments will be conducted with patients standing 1.25 m away from a 1.80 m×1.20 m screen. fNIRS recordings will start with a 20-s rest period. The paradigm includes standing balance tasks on hard and soft ground, repeated six times for each condition. Each mission is 20 s in length. Patients will watch a video for 20 s, stand on the floor for 20 s, and then rest for 20 s. The tasks of standing on hard ground and standing on soft ground will be given in a random order for each participant. The order of the tasks was randomized to eliminate

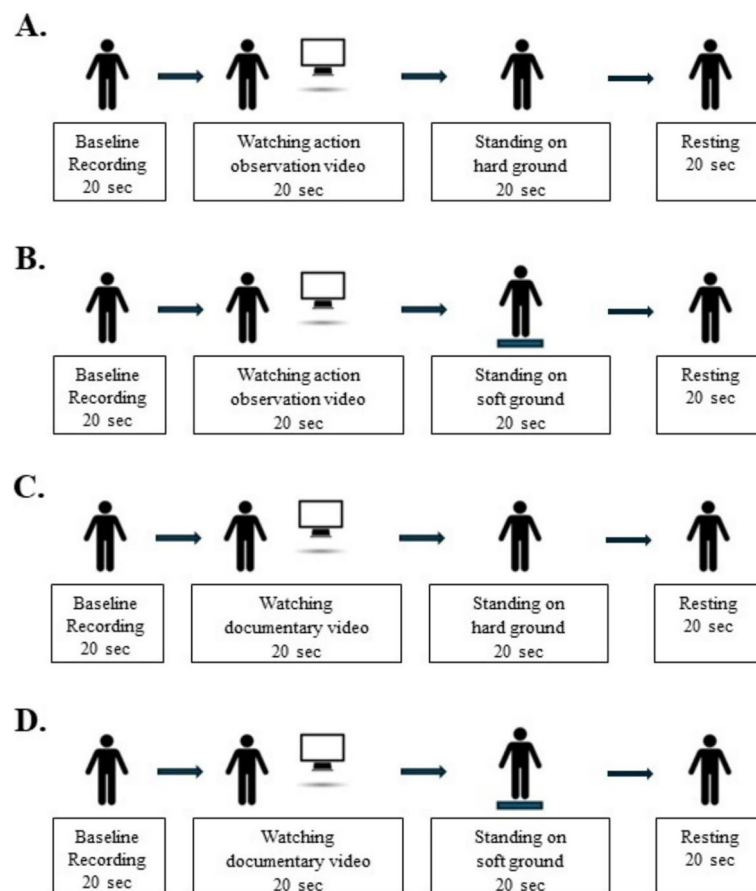


Fig. 4 fNIRS experiment paradigm. **A** Action observation therapy group fNIRS recording on hard ground; **B** action observation therapy group fNIRS recording on soft ground; **C** control group fNIRS recording on hard ground; **D** control group fNIRS recording on soft ground (sec: seconds)

the effects of learning and fatigue. Two separate videos will be prepared for two groups. While patients in the action observation therapy group will watch an action observation video, patients in the control group will watch a documentary film that does not contain motor movements (Fig. 4).

Post-rehabilitation assessment and fNIRS recordings

The tests and fNIRS recordings performed before rehabilitation will be repeated similarly. The procedure is summarized in Fig. 1.

Data analysis approach

This study design will produce a relatively large hemodynamic and motor data set:

Data on motor functions before and after rehabilitation for two groups
fNIRS data before and after rehabilitation for two groups (action observation therapy group and control group)

fNIRS data for two tasks (on hard ground and soft ground)

fNIRS data for two videos (action observation video and control video)

fNIRS data for three conditions (rest, video, balance on different surfaces)

Score changes in motor function tests that will occur following each application (action observation and control)

Correlation between hemoglobin amount and motor and balance test scores

Correlation of changes between hemoglobin amount change and motor scores after treatment

Data will be recorded, anonymized, and stored during the experiment without processing or creating any output. All manually scored tests will be scored by a specialist physiotherapist and entered independently. fNIRS data quality will be checked before, during, and after recording. As soon as each patient's rehabilitation process is completed, analyses will be carried out, and the

patient's relatives will be informed about the rehabilitation effect. All data are expected to be collected for group analysis, and the study will be completed.

Analysis of fNIRS data

Regarding the fNIRS technique, several methods and analysis strategies were identified to evaluate the effects of AOT. First, the amount of HbO and Hb that will increase or decrease in fNIRS data following therapies will be determined. In addition, it should be remembered that the total hemoglobin amount can be calculated.

In this study, the primary neuroimaging outcome will be the mean change in HbO concentration during task performance, measured within predefined regions of interest (ROIs), primarily motor-related cortical areas including the supplementary motor area, premotor cortex, and primary motor cortex. The statistical analysis will be based on average activation amplitude measures. This approach was chosen to directly assess hemodynamic responses related to motor function improvements following the intervention. The analysis will include a general linear model approach and evaluate changes in different groups by determining pre- and post-treatment changes. Additionally, fNIRS data collected during the mission (while watching different videos and from various backgrounds) will be evaluated, and these hemodynamic data will be considered the primary dependent variables.

fNIRS data will be preprocessed using Homer 3 [35, 36], an open-source, Matlab-based software package. The steps below align with the latest fNIRS data processing recommendations [36–39]. In the fNIRS analysis, noisy channels with very low optical density (coefficient of variation > 10%) will be excluded (enPruneChannels). The remaining raw optical density data will then be converted to changes in optical density (OD) (hmrIntensity2OD). Motion artifacts will then be corrected using a Wavelet transform [40]. hmrR_MotionCorrectWavelet (iqr = 1.50) and hmrR_MotionArtifactByChanenel (tMotion = 0.5, tMask = 1.0, STDEVthresh = 20.0, AMPthresh = 5.0) will be used to correct and detect motion artifacts, respectively. Trials with movement artifacts occurring 2 s before the presentation of the stimuli and 20 s after the presentation of the stimuli will be excluded from the analysis. The signal will then be filtered with a high-pass cut-off frequency of 0.01 Hz (hmrBandpassFilt) to account for low-frequency aberrations and filtered with a low-pass cut-off frequency of 0.5 Hz (third-order Butterworth filter) to remove the effects of self-generated physiological components [37]. The preprocessed optical density data of both wavelengths will then be converted into oxygenated (HbO) and deoxygenated hemoglobin (Hb) concentration changes using the modified Beer–Lambert

equation ($\text{ppf} = 1.0 \pm 1.0$) [41]. The age- and wavelength-adjusted differential path length factor (DPF) required for this equation will be calculated according to Scholkmann and Wolf (2013). To calculate the hemodynamic response function (HRF), it will be deconvolved using a general linear model (GLM) based on ordinary least squares regression over some time of –5 to 20 s. hmrR_GLM function (trange = –2.0 14.0, glmSolveMethod = 1, idxBasis = 1, paramsBasis 1.0 1.0, rhoSD_ssThresh = 40.0, flagNuisanceRMethod = 2, driftOrder = 3, c_vector = 0) will be used. To minimize the non-brain component due to superficial tissue, the average signal from the channels with the shortest source-detector distances will be used in our setup (rhoSD_ssThresh = 40, flagNuisanceRMethod = 2) [42]. The HRF will be modeled using a sequential set of Gaussian HRF basis functions with a standard deviation of 0.5 s and means separated by 0.5 s. A third-order polynomial will be included to account for baseline drift. The HRFs recovered for each channel (HbO and Hb) will be baseline corrected by subtracting the average of the HRF between –5 and 0 s from each data point.

Finally, hemodynamic response functions (HbO and Hb) will be determined for each channel 2 s before the onset of the stimulus and 15 s after the onset of the stimulus, and the average HbO value in this period will be taken into statistical analysis [31, 33, 37]. The main fNIRS metric will be the average activation amplitude (i.e., the mean HbO signal) during a defined time window (2 s before to 15 s after stimulus onset), compared between pre- and post-intervention sessions. This approach allows for direct quantification of hemodynamic response changes attributable to the intervention. In addition to this primary metric, laterality index and functional connectivity (e.g., inter-regional temporal correlation of HbO signals) may be explored as secondary outcomes, if data quality and signal-to-noise ratio allow. All fNIRS data will be preprocessed and analyzed using Homer3, with the pipeline previously described. The GLM will be applied to extract the HRF per channel, and average HbO changes within our regions of interest will be extracted and entered into the statistical analysis.

Exercise training

Patients will be randomly divided into two groups. Randomization was performed using a computer-generated random number sequence created with Microsoft Excel. Each participant was assigned a random number, and allocation to either the action observation therapy group or the control group was carried out in a 1:1 ratio according to this sequence. In this study, AOT will be applied to improve balance. The videos to be used in the study will be designed to visually represent selected actions

appropriate for the participants' ages, subdivided into simple motor tasks arranged in order of increasing complexity. Considering that the effectiveness of AOT may be enhanced by having the observed model be of a similar age to the children receiving the treatment, the videos will be prepared accordingly. The therapist will verbally explain the program to be used to both groups. Children with CP will watch a video on a computer screen and be placed 1 m in front of a chair where they can sit comfortably. They will not be allowed to move while watching the video. Children with CP will watch the video of the exercise presented by the therapist and then perform the exercise. The viewing time for each exercise is 3 min; after watching, they must do the exercise in the video for 3 min. The exercises will be explained to the individual who will do the exercise in the control group. The control group will perform the same exercise program for the same duration as the intervention group. The control group will be shown a documentary film that does not contain motor movements. After a 3-min monitoring period, each exercise will be performed for 3 min. To increase the effectiveness of action observation training, patients will watch the video in a quiet and noiseless place at a designated time. The treatment program will be applied for 30 min per day, 5 days a week for 3 weeks. The exercise program will be communicated to the patients online and will be followed online every day. Treatment will begin within the first 48 h after the evaluation. After the applications, the assessment will be repeated at the beginning of the session. Both groups will follow the same exercise program.

During the treatment process, it will be stated that additional therapies outside the recommended program should not be applied, and this situation will be meticulously monitored. Another important element in exercises performed at home is safety, especially during balance exercises, since the risk of falling is high; a parent or adult must be present near the child. The exercise area should have a non-slip and safe surface, and there should be no sharp or hard objects in the environment. In addition, the child should wear suitable non-slip shoes or socks. The exercises should be applied in a controlled manner without being overly challenging. It is also of great importance for parents to have basic first aid knowledge in terms of rapid intervention in emergencies. All these points will be meticulously paid attention to; thus, both the effectiveness of the treatment will be preserved and the safety of the child will be ensured.

Exercise program

Week 1:

- Standing on a soft surface with eyes open and arms free, maintaining balance on both feet
- Standing on a soft surface with eyes closed and arms free, maintaining balance on both feet
- Standing on a firm surface on one foot with eyes open
- Standing on a firm surface on one foot with eyes closed

Week 2:

- Standing on a firm surface with feet together
- Standing on a firm surface in tandem position
- Weight shifting to the right and left lower extremities
- Taking a forward step from a standing position

Week 3:

- Standing on a soft surface with feet together
- Standing on a soft surface in tandem position
- Taking steps in different directions from a standing position
- Kicking a ball with the foot

Statistical analysis

Statistical analyses will be pre-specified based on the level of measurement and data distribution. Group comparisons will primarily be conducted using the independent-samples *t*-test for normally distributed continuous variables and the Mann–Whitney *U* test for non-normally distributed variables. Categorical variables will be compared using the chi-square test. The assumptions for each test (e.g., normality and homogeneity of variance for *t*-tests) will be assessed using appropriate diagnostics such as the Shapiro–Wilk test and Levene's test.

To address potential issues with multiple comparisons across outcome variables (e.g., motor scores, balance metrics, and hemodynamic changes), a false discovery rate (FDR) correction will be applied when applicable.

For continuous outcome measures involving covariates (e.g., age, baseline performance), analysis of covariance (ANCOVA) or multiple regression models will be used, as pre-specified in the analysis plan. Effect sizes (e.g., Cohen's *d* or partial eta-squared) will be reported alongside *p* values to provide an estimate of practical significance.

Exploratory analyses will only be conducted if unexpected patterns arise and will be clearly distinguished from the primary confirmatory analyses. These analyses will be reported as hypothesis-generating and interpreted with appropriate caution.

Sample size was determined based on a priori power analysis using estimated effect sizes from relevant literature. All analyses will be conducted using SPSS with a significance threshold set at $\alpha=0.05$ (two-tailed), unless otherwise corrected for multiple comparisons.

Discussion

This study evaluates the effect of AOT in patients with CP in terms of brain activation and motor symptoms. It aims to reveal static and dynamic balance functions with fNIRS data. In addition, fNIRS seeks to uncover the regional blood flow changes in the brain that are likely to occur due to treatment.

AOT involves observing specific actions performed by others and then matching them with an action representation based on their characteristics. Repeated application of the observed actions after this observation-matching facilitates motor learning [24]. AOT has the potential to become a routine approach in the rehabilitation of children with CP [43]. It is reported that by observing actions, an internal representation of the observed movement can be evoked in the brain, strengthening the formation of motor memory and facilitating subsequent motor performance. Thus, it affects the motor learning mechanism [6]. Although it is generally associated with motor areas, especially the ventral premotor area, supplementary motor area, temporal and parietal regions seem closely related to motor learning and action observation [24].

In investigating the action observation network, fNIRS can be easily preferred in infants and children. fNIRS is a neuroimaging technique with high motion tolerance compared to fMRI and EEG. In addition to allowing simultaneous recording with motor function, this feature makes fNIRS a more suitable option for active groups of babies and children [22, 44]. Despite these advantages, few studies evaluate hemodynamic activity during motor tasks in children with CP [45, 46]. HbO concentration occurring in the supplementary motor area, precentral gyrus, postcentral gyrus, and superior parietal lobule indicates the degree of cortical involvement during walking. A study shows that children with CP need more cortical activity to control their walking [21]. A study evaluating the effect of progressive testing of lower extremity functional muscle strength on prefrontal cortex hemodynamic activity in children with CP stated that hemodynamic activity was low and showed different development compared to typically developing children [47]. It has been shown that there is a significant increase in activation in the prefrontal cortex with fNIRS after 12 sessions of walking training using Lokomat in 8 children with CP [48]. These studies indicate that fNIRS has essential potential in evaluating hemodynamic activity

during motor tasks in children with CP and that fNIRS may be an important technique to demonstrate the treatment effect in rehabilitation protocols.

There are some limitations in the study design. The groups were considered patients with a certain level of CP who only had lower extremity problems. This will provide homogenization in terms of data but will not provide data on the entire disease group. Furthermore, since only children with CP who had lower extremity problems were included in the study, the study evaluation parameters were limited to motor balance scales. In addition to these, cognitive and imagery evaluations can be added to the inclusion criteria. Comparison with healthy age-matched children may provide more comprehensive information. The duration of the task will be adjusted to be as short as possible, considering the potential fatigue of the patients and the difficulty of the task. The study was not designed as a masked (blinded) study. However, designing future studies with blinded evaluators (i.e., using a masked assessor) will help eliminate possible biases. Recording fNIRS data only from the motor and premotor areas may be sufficient, considering the motor network. In future studies, the montage can be designed to record from more extensive areas. A disadvantage of the fNIRS technique is that subcortical structures cannot be recorded. With this study, activation in subcortical structures with essential motor task functions will not be revealed. However, thanks to the fNIRS technique's resistance to motion noise, it provides vital information about motor activation by measuring changes in hemoglobin levels in the brain simultaneously with motor tasks.

In conclusion, this study aims to comprehensively evaluate the effect of the AOT protocol and its hemodynamic and motor effects in children with cerebral palsy. Additionally, this study aims to determine the impact of AOT using motor and balance tests and the fNIRS technique and to create a new protocol.

Supplementary Information

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Additional file 1. SPIRIT checklist

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Authors' contributions

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. Conceptualization: MO, LH, FKM. Methodology: MO, MS, LH, FKM. Software: MO, MS. Writing—original draft preparation: MO, MS. Writing—review and editing: MO, MS, LH, FKM. Visualization: MO, MS. Supervision: MS, LH, FKM.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors have no conflicts of interest or any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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