Study Design and Participants

This study was conducted as a randomized, sham-controlled, single-blind clinical trial to investigate the combined effects of Botulinum Toxin Type A (BTX-A) and Mirror Therapy (MT) on upper extremity spasticity in stroke patients. Ethical approval was obtained from the Institutional Review Biruni University Clinical Research Ethics Committee and all participants provided written informed consent prior to enrollment in accordance with the Declaration of Helsinki (2015-KAEK-80 23 50).

Participants were selected from the neurological rehabilitation clinic of the physical medicine and rehabilitation unit at Biruni University Hospital. Eligible patients were those diagnosed with unilateral ischemic stroke and scheduled to receive BTX-A injections for upper extremity spasticity management.

Inclusion criteria were as follows:

- Age ≥ 18 years
- Diagnosis of unilateral ischemic stroke confirmed by neuroimaging (MRI or CT)
- At least 3 months post-stroke to ensure chronic phase status
- Routine follow-up under the care of the same clinician who determined BTX-A treatment indication based on established clinical guidelines
- Mini-Mental State Examination (MMSE) score > 24 to ensure adequate cognitive function for participation
- Fugl-Meyer Assessment (FMA) upper extremity motor score < 55, indicating moderate to severe motor impairment
- Modified Ashworth Scale (MAS) score between 1 and 4 in the affected upper extremity, indicating presence of spasticity

Exclusion criteria:

- Prior BTX-A or other neurotoxin injections to the affected upper extremity within the last 6 months
- Severe cognitive impairment or psychiatric disorders interfering with communication or compliance
- Concurrent participation in other experimental rehabilitation or pharmacological trials

- Visual or motor deficits severe enough to prevent effective engagement with Mirror Therapy
- History of multiple strokes or bilateral brain lesions
- Presence of neuromuscular diseases affecting the unaffected upper extremity
- Stroke etiology other than ischemic (e.g., hemorrhagic stroke, traumatic brain injury)

A total of 43 participants were initially enrolled between May 2024 and February 2025 from the neurological rehabilitation clinic of the Physical Medicine and Rehabilitation Department at Biruni University Hospital. Following baseline evaluations and BTX-A injections, participants were randomly allocated into two groups: 22 patients in the Mirror Therapy (MT) group and 21 patients in the Sham Therapy (SHA) group. Permuted block randomization was employed to ensure balanced group sizes and reduce selection bias. The randomization sequence was generated using a computer-based random number system and was implemented by an independent researcher not involved in patient assessment or treatment. Group allocation was concealed using sealed, opaque envelopes until the point of assignment.

In the MT group**,** 4 participants were excluded due to irregular attendance (<75% session compliance), 2 participant withdrew their informed consent, and 1 were hospitalized for unrelated medical conditions requiring inpatient treatment. In the SHA group, 4 participants failed to adhere to the intervention frequency, and 2 were excluded because they received additional pain management therapies during the follow-up period.

Consequently, 15 participants in each group (n = 30 total) completed the full 6-month intervention and follow-up assessments and were included in the final statistical analysis. The overall participant flow, including recruitment, allocation, and attrition, is illustrated in.

The study was conducted according to the approved protocol and completed as planned without early termination. The follow-up period for each participant lasted six months after BTX-A injection, and all post-treatment evaluations were completed by March 2025**.**

All intervention sessions were delivered by the same licensed physical therapist specialized in neurorehabilitation, ensuring procedural consistency across both groups. Adherence to the

intervention protocol was closely monitored by weekly attendance tracking and therapist supervision to maintain treatment fidelity.

Participants were allocated as follows:

- **Experimental Group (Group I, n=15):** Received mirror therapy in addition to conventional rehabilitation.
- **Control Group (Group II, n=15):** Received a sham intervention alongside conventional rehabilitation. In this group, a transparent glass panel was used in place of a mirror, and patients were instructed to observe the reflection of their paretic extremity, thereby eliminating the visual illusion effect central to mirror therapy.

A single-blind study design was implemented to minimize bias. Neither the participants nor the outcome assessors were aware of group assignments. Both the mirror therapy and sham interventions were administered by a licensed physical therapist experienced in neurorehabilitation, who was not involved in data analysis or outcome evaluation.

Evaluation Criteria

Treatment efficacy was assessed at two time points: baseline (prior to BTX-A injection) and six months post-injection. The following standardized clinical instruments were employed to evaluate motor function, spasticity, and muscle strength:

Motor Function: Assessed using the Brunnstrom Recovery Stages, with separate scoring for the hand and upper extremity. This scale provides a stage-based evaluation of motor recovery following stroke, ranging from flaccidity (Stage I) to near-normal movement patterns (Stage VI).

Spasticity**:** Evaluated using the Modified Ashworth Scale (MAS), which quantifies resistance during passive soft-tissue stretching. The MAS scores range from 0 (no increase in muscle tone) to 4 (rigid in flexion or extension), offering a reliable measure of spasticity severity.

Overall Motor Performance: Measured with the Fugl-Meyer Assessment for Upper Extremity (FMA-UE), a validated and widely used tool that evaluates motor function, coordination, and

reflexes in the paretic arm. Scores range from 0 to 66, with lower scores indicating more severe impairment.

Muscle Strength: Quantified using a handheld dynamometer (Jamar® or equivalent) for both the paretic and non-paretic upper extremities. Grip strength (in kilograms) was recorded as the maximum value of three consecutive trials, with standardized positioning to ensure consistency.

All assessments were conducted by a trained physiotherapist (N.T.) blinded to group allocation. Standardized testing protocols were followed across all time points to ensure inter-session reliability.

Intervention Protocol

Following BTX-A injection, all participants in both groups underwent a standardized conventional upper extremity rehabilitation program for a duration of 6 months, with sessions conducted three times per week. The rehabilitation protocol included stretching exercises, passive and active-assisted range of motion exercises, coordination and balance training, as well as functional task-oriented activities. All sessions were conducted in a one-on-one setting by experienced physiotherapists.

Experimental Group (Group I): Mirror Therapy Application

In the experimental group, mirror therapy (MT) was administered in addition to conventional rehabilitation. The MT protocol was implemented as follows:

- Participants were seated upright with a large mirror placed in the midsagittal plane of the body, such that the reflection of the non-paretic extremity appeared to replace the paretic extremity.
- Movements were performed with the non-paretic extremity while patients observed the mirrored image, creating the visual illusion that the paretic extremity was moving.
- Mirror therapy was applied three times per week, with each session lasting 30 minutes, for a total of 60 sessions.
- Exercises included functional movement patterns such as finger flexion and extension, thumb abduction–adduction, and thumb opposition movements, as well as wrist flexion/extension, forearm pronation/supination, elbow flexion/extension, and simple grasp-and-release activities.

- The sequence of movements was progressively adapted to the participant's functional level, starting with isolated distal motions (finger and thumb exercises) and advancing toward combined and proximal joint movements (wrist and elbow). Patients were continuously encouraged to focus on the mirrored image to reinforce the visual illusion of symmetrical movement.

Control Group (Group II): Sham Intervention

The control group received a sham intervention designed to mimic the physical setup of mirror therapy but without inducing the visual illusion:

- A transparent glass panel was used in place of a mirror, and participants were asked to observe the reflection of their paretic extremity directly through the glass.
- This setup was intended to replicate the environment of MT without stimulating the motor cortex through visual feedback.
- The frequency, duration, and total number of sessions matched those of the experimental group (3 sessions/week, 30 minutes per session, 60 sessions in total).

All participants initiated their rehabilitation and mirror/sham therapy sessions two weeks after the BTX-A injection, coinciding with the onset of the toxin's maximal clinical effect. Adherence to the intervention protocol was strictly monitored throughout the study period.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Shapiro-Wilk test. As the data were not normally distributed, non-parametric tests were employed for all comparisons. Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were presented as frequencies and percentages. Between-group comparisons at baseline and post-treatment were conducted using the Mann–Whitney U test for continuous variables and the Chi-square test for categorical variables. To evaluate within-group changes from pre- to post-treatment, the Wilcoxon signed-rank test was used. In addition, Spearman's rank-order correlation coefficient (ρ) was calculated to assess the relationships between changes in motor function, spasticity, and muscle strength. A two-tailed p-value < 0.05 was considered statistically significant for all analyses. No corrections for multiple comparisons were applied, as the primary outcomes were pre-specified.

A priori sample size estimation was performed using G*Power software (Version 3.1), assuming a two-tailed test with an effect size of $d = 0.62$, $\alpha = 0.05$, and power $(1-\beta) = 0.80$. A minimum total sample size of 28 participants (13 in one group and 15 in the other) was deemed sufficient to detect statistically meaningful between-group differences.