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ORIGINAL ARTICLE

# Serum Brain-Derived Neurotrophic Factor (BDNF) Enhancement Through Task-Specific Exercises and Transcranial Simulation: A Randomised Pilot Controlled Study in Stroke Survivors

**MUHAMMAD UMAR A[1,2], SHARIFUDIN MA[1]*, RAJ NB[3], AHMAD AA[4]**

*[1]Department of Orthopaedics, Traumatology and Rehabilitation, Faculty of Medicine, Universiti Sultan Zainal Abidin, Jalan Sultan Mahmud, Kuala Terengganu, Terengganu, Malaysia*
*[2]Department of Human Physiology, Federal University Dutse, Jigawa, Nigeria*
*[3]School of Rehabilitation Science, Faculty of Health Sciences, Universiti Sultan Zainal Abidin, Kuala Nerus, Terengganu, Malaysia*
*[4]Department of Physiotherapy, Bayero University, Nigeria*

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## *ABSTRAK*

*Kajian rintis ini bertujuan untuk menilai kesan rehabilitasi selama 4 minggu yang melibatkan latihan tugasan khusus (TSE), stimulasi arus terus transkranial (tDCS), dan gabungannya terhadap peningkatan aras faktor neurotropik yang berasal dari otak (BDNF) dalam kalangan penghidap strok ambulatori. Sejumlah 60 pesakit strok ambulatori telah dibahagikan secara rawak kepada empat kumpulan intervensi; (i) fisioterapi intervensi kawalan (CIP); (ii) gabungan TSE, tDCS, dan CIP; (iii) TSE dan CIP; dan (iv) tDCS dan CIP. Tahap serum BDNF dinilai menggunakan kit Quantikine ELISA. Perbezaan yang signifikan secara statistik dalam tahap serum BDNF diperolehi di antara kumpulan-kumpulan tersebut ($F=58.04$, $p=0.001$). Analisis post-hoc Bonferroni menunjukkan tiada perbezaan yang signifikan dalam tahap serum BDNF antara kumpulan kawalan dan TSE + CIP ($p=1.000$). Perbezaan yang signifikan secara statistik dalam serum BDNF dilaporkan antara kumpulan kawalan, tDCS + CIP, dan TSE + tDCS + CIP pada $p<0.001$. Kajian rintis ini menunjukkan kesan yang ketara intervensi terhadap tahap serum BDNF dalam pesakit strok. Gabungan TSE, tDCS, dan CIP menunjukkan keberkesanan dalam meningkatkan tahap BDNF pesakit. Tiada perbezaan yang signifikan dalam tahap*

**Address for correspondence and reprint requests**: Assoc. Prof. Dr. Mohd Ariff bin Sharifudin. Department of Orthopaedics, Traumatology and Rehabilitation, Faculty of Medicine, Universiti Sultan Zainal Abidin (UniSZA), Medical Campus, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Terengganu, Malaysia. Tel: +609-6275630/ +6019-3352499 Email: ariffsharifudin@unisza.edu.my / dr.ariff.s@gmail.com

*BDNF dapat dikenalpasti antara kumpulan kawalan dan TSE + CIP. Penemuan ini memberikan harapan untuk memperhalusi strategi penyelidikan dan rehabilitasi dalam pesakit strok untuk mendorong neuroplastisiti berkaitan BDNF.*

*Kata kunci: Faktor pertumbuhan neurotropik; kajian rintis; korteks motor utama, peningkatan neuroplastisiti; rehabilitasi strok; serangan cerebrovascular*

## ABSTRACT

This pilot study aimed to evaluate the effect of four-week rehabilitation regimens involving task-specific exercises (TSE), transcranial direct current stimulation (tDCS), and their combination on enhancing serum brain-derived neurotrophic factor (BDNF) levels in ambulatory stroke survivors. A total of 60 ambulatory stroke patients were randomly assigned to four intervention groups: (i) control intervention physiotherapy (CIP); (ii) combination of TSE, tDCS, and CIP; (iii) TSE and CIP; and (iv) tDCS and CIP. Serum BDNF levels were assessed using a Quantikine ELISA kit. A statistically significant variance in serum BDNF levels was observed among the groups ($F=58.04$, $p=0.001$). Bonferroni's post-hoc analysis revealed no significant difference in serum BDNF levels between the control and TSE+CIP groups ($p=1.000$). A statistically significant difference in serum BDNF was reported between the control, tDCS+CIP group, and TSE + tDCS + CIP group at $p<0.001$. The pilot study underscores the considerable influence of interventions on serum BDNF levels in stroke survivors. The combined regimen of TSE, tDCS, and CIP demonstrated efficacy in enhancing patients' BDNF levels. No significant difference in BDNF levels was discerned between the control and TSE+CIP groups. These findings hold promise for refining research and rehabilitation strategies in stroke patients to promote BDNF-related neuroplasticity.

Keywords: Cerebrovascular accident; neuroplasticity enhancement; neurotropic growth factors; pilot study; primary motor cortex; stroke rehabilitation

## INTRODUCTION

Stroke, also known as cerebrovascular accident (CVA), is a neurological condition resulting from cerebrovascular injury, leading to either partial or complete brain dysfunction due to the blockage or rupture of cerebral blood vessels (Choi 2022; Srinayanti et al. 2021). After a stroke, substantial neuroplasticity occurs, contributing to spontaneous recovery of sensory, motor, and cognitive functions (Murphy & Corbett 2009). It has been suggested that elevated cortical excitability, coupled with changes in synaptic plasticity like long-term potentiation-like modulation, increased calcium currents, and activation of neurotrophic factors

within the affected hemisphere, are key mechanisms facilitating post-stroke recovery (Murphy & Corbett 2009). Stroke-related complications, such as sensorimotor and cognitive impairments, pose a significant burden on survivors, their families, and society, resulting in substantial global adult disability rates (Choi 2022).

Stroke survivors experience a period of re-adjustment and strive to regain normalcy following hospitalisation (Baune & Aljeesh 2006). However, when they realise their limitations in performing daily activities and the loss of their routine, their home life becomes challenging (Yoo & Kim 2015). This is when the post-stroke consequences are felt (Baune & Aljeesh 2006). Stroke survivors tend to compare their current lives with their pre-stroke existence to revert to their former selves. Research indicates that stress experienced by stroke survivors' post-hospital discharge is directly associated with the presence of depressive symptoms and their level of functional independence. Those with lower functional independence and more severe depressive symptoms encounter higher levels of stress upon returning home (Baune & Aljeesh 2006; Kong & Yang 2006). The persistence of psychological stress in stroke survivors can detrimentally affect neural function. Post-stroke events typically lead to neural plasticity, involving the release of various neurotrophic growth factors, including brain-derived neurotrophic factor (BDNF). BDNF plays a role in modulating activity-dependent synaptic plasticity in the human motor cortex (Alsharidah et al. 2018). It promotes the proliferation, survival, and differentiation of neurons in both the central and peripheral nervous systems, as well as triggering anti-apoptotic mechanisms post-stroke, reducing infarct size and secondary neuronal cell death (Aydemir et al. 2006). BDNF concentrations tend to decrease with the stroke's severity, originating from various sources, including the bloodstream and platelets (Lommatzsch et al. 2005).

Rehabilitation programs are designed to promote neural plasticity and enhance the release of growth factor proteins, thereby improving the function of affected limbs. By directing patients to concentrate on movements of the unaffected side, these programs stimulate neural plasticity in the affected area (Stevens & Stoykov 2003). Hypoxia resulting from stroke disrupts normal neuronal function, leading to the inability to maintain typical trans-membrane ionic gradients and triggering apoptotic and necrotic cell death pathways (Laste et al. 2012). This damage often disrupts normal patterns of synaptic excitatory activity in both the immediate vicinity of the infarct and in distal regions functionally linked to the site of injury (Bolay et al. 2000; Bütefisch et al. 2008; Carmichael et al. 2001; Carmichael et al. 2004). Studies have indicated that several mechanisms contribute to stroke recovery, including heightened cortical excitability, alterations in synaptic plasticity such as long-term potentiation (LTP)-like modulation, increased calcium membrane influx, and activation of neurotrophic factors within the affected hemisphere

(Murphy & Corbett, 2009).

Exercise acts as a physical intervention that triggers the formation of fibronectin type III domain-containing 5 (FNDC5) (Constans et al. 2021). FNDC5, a myokine, is upregulated in the hippocampus during training, stimulating the activation of BDNF and other neuroprotective genes in both human and mouse hippocampi, thereby promoting neuroplasticity. Stroke patients often experience increased neuroinflammation, impacting neuroplasticity processes in the core lesion, penumbra, and remote areas (Jones & Adkins 2015; Pin-Barre et al. 2014; Pin-Barre et al. 2018; Tennant 2014). Exercise plays a pivotal role in mitigating neuroinflammation by downregulating genes associated with the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), which regulates the subgranular zone (SGZ) niche of stem cells (Hugues et al. 2021). Disruption in GABA function may elevate BDNF levels, thereby mediating neurogenesis, alongside alterations in the expression of potassium chloride cotransporter (KCC2) and sodium/potassium/chloride cotransporter type 1 (NKCC1), particularly affected by decreased cerebral blood flow in stroke. During exercise, skeletal muscles communicate with various organs, including the brain. Accumulation and release of substances like lactate during active sessions have been strongly correlated with increased serum BDNF levels and motor cortex excitability (Hugues et al. 2021).

Task-specific exercise (TSE) involves patients engaging in context-specific motor tasks and receiving feedback (Rizos et al. 2010). This approach emphasises enhancing performance in functional tasks through goal-directed practice and repetition (Vinogradov et al. 2009). Increasing evidence suggests neural plastic changes linked with TSE, particularly in specific skill learning, aligning with a learning-dependent model of neural plasticity (Vinogradov et al. 2009). Conversely, transcranial direct current stimulation (tDCS) represents a noninvasive technique for modulating neural excitability in targeted brain regions. Researchers hypothesised that anodal tDCS enhances synaptic plasticity by inducing membrane depolarisation, akin to neuronal activation, thereby promoting BDNF transcription through epigenetic mechanisms. Epigenetic alterations triggered by tDCS facilitate increased transcription of BDNF precursor and histone acetylation (Aid et al. 2007; Karpova 2014; Pruunsild et al. 2011). Anodal tDCS effectively augments neural excitability in the target brain region, whereas cathodal tDCS leads to membrane hyperpolarisation. This technique involves the application of low-amplitude electrical currents (1-2mA) via electrodes positioned on the scalp over the targeted brain region (Cavaleiro et al. 2020).

Despite numerous reports highlighting the effectiveness of tDCS and TSE in stroke rehabilitation, most of the studies did not provide specific conclusions regarding the impact of these interventions on post-stroke serum BDNF. Thus, this pilot study sought to determine the

solitary and combined effects of these interventions on serum BDNF levels in stroke survivors.

## MATERIALS AND METHODS

In this pilot randomised controlled study, a total of 60 participants were recruited after obtaining written informed consent. The eligibility criteria included participants who experienced their first-ever stroke, had no history of drug abuse, presented with no other medical or psychiatric diseases, and either gender aged between 24 and 65 years, exhibited no visual or auditory deficits, able to respond to verbal commands, and were not hospitalised during the course of the study. The participants must also score 22 or higher on the Mini-Mental State Examination (MMSE).

Participants who were actively engaging in exercise or had received proprioceptive neuromuscular facilitation (PNF) treatment for less than three months prior to the study, had recurrent strokes, fractures, amputations, or were unable to walk freely or with an assistive device, were excluded from the study. Those who were on antidepressant medications were also excluded. However, anti-hypertensive and anti-diabetic drugs were not part of the exclusion criteria. Additionally, we also excluded participants who were participating in other research at the time of the current study was conducted.

The study was conducted at Imamu Wali General Hospital in Kano, Nigeria after obtaining approval from the Health Research Ethics Committee of the Ministry of Health, Kano State of Nigeria (NHREC/17/03/2018). The health research ethics committee approval was issued on the 12th of April 2023. The sample size was determined using the formula: n = In (1-ϒ)/In (1-π), where n represented the sample size, ϒ denoted the confidence level set at 0.095, and  signified the problem probability set at 0.05. The calculated result, n = In (1-0.095)/ In (1-0.05), equated to 58.4, which was rounded to 60 participants allocated across four groups. The participants were assigned to the four groups using a simple random sampling method. Each participant meeting the study criteria received a computer-generated number. The group consisted of (i) those undergoing control intervention physiotherapy (CIP) (n=15), (ii) participants receiving a comprehensive treatment regimen, including TSEs, tDCS, and conventional physiotherapy (TSE + tDCS + CIP, n=15), (iii) participants engaging in TSEs alongside conventional physiotherapy (TSE + CIP, n = 15), and (iv) those receiving tDCS combined with conventional physiotherapy (tDCS + CIP, n=15). This process ensured an equal and unbiased opportunity for selection among eligible participants. Figure 1 provided a visual summary of the randomisation and group assignment process.

The study procedures were thoroughly communicated to the participants. Both the patients and the investigators, including physiotherapists and laboratory scientists, were blinded to the assigned intervention and assessment groups.

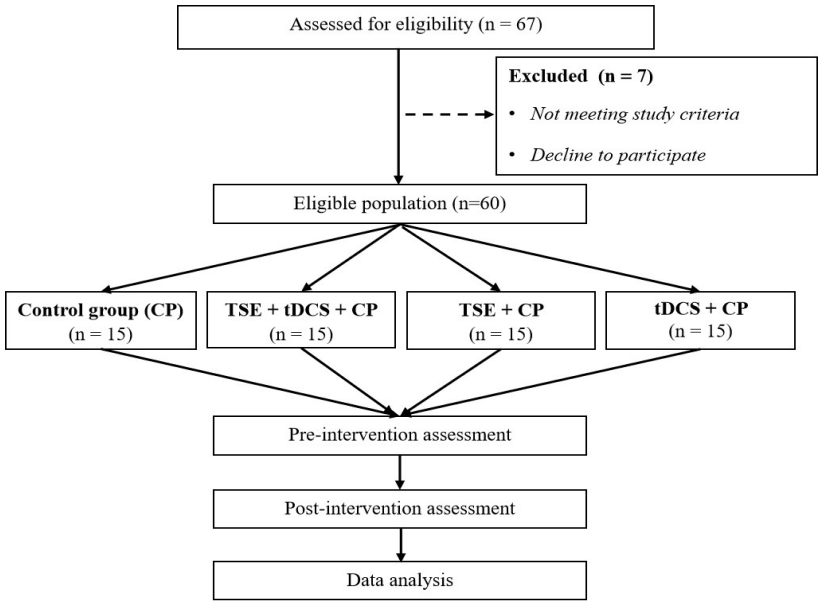


FIGURE 1: Flow chart of the study (tDCS: transcranial direct current stimulation; TSE: task-specific exercises)

Baseline data of the participants were collected, and the respective interventions were administered three times daily over four weeks. In the combined TSE + tDCS +CIP group, participants received two additional components to CIP: 20 minutes of anodal tDCS, followed by 10 sets of TSE, along with 20 minutes of the CIP intervention. The socio-demographic characteristics of the participants were documented using a data collection sheet with pertinent variables. Blood pressure was measured twice using validated and calibrated mercury and digital sphygmomanometers. The average was recorded for systolic and diastolic readings. Body mass index (BMI) was calculated using participants' weight and height measured with a digital scale and a calibrated wall. The BMI formula, BMI = weight of the participant in kg/(height of the participant in meters)$^2$, was applied using the recorded values.

## Assessment of Serum Brain-Derived Neurotrophic Factor (BDNF)

Serum BDNF levels were assessed twice, before and after four weeks of interventions. Five millilitres of blood were collected from the cubital vein of patients in the morning between 8:00 AM and 9:00 AM to minimise circadian effects. The samples were centrifuged at 2000xg for 20 minutes, separated, and stored at -80°C for subsequent analysis. Measurement of serum BDNF levels was performed using a commercially available Human BDNF ELISA Kit (Sunlong Biotech, China) following the manufacturer's instructions. This ELISA kit utilised the Sandwich-ELISA

method, where standards or samples were added to specific microplate wells and combined with specific antibodies. A Horseradish Peroxidase (HRP)-conjugated antibody specific for BDNF was also added to each well and incubated. After washing away free components, wells containing BDNF and HRP-conjugated BDNF antibodies produced a colour change from blue to yellow after adding the stop solution. The amount of BDNF in the test solution was proportional to the colour generated in the redox reaction.

## Transcranial Direct Current Stimulation (tDCS)

Prior to each tDCS session, a baseline assessment of serum BDNF levels and patients' socio-demographics were conducted three days in advance. Each session lasted for a duration of 20 minutes. Before commencing stimulation, the functional deficits of patients were evaluated, and thorough explanations regarding therapy plans were provided to both patients and caretakers.

The positioning and electrode montage for stimulating the primary motor cortex (PMC) were determined using the International 10-20% electrode encephalography (EEG) system, which indicated the relationship between electrode location and the corresponding brain area beneath the scalp. Anodal tDCS was administered using a Medisystems tDCS DC-Stimulator Plus device, with the anodal electrode serving as the active electrode and the cathodal electrode as the reference electrode. Two 5x5 cm non-metallic surface electrodes, wrapped in moist saline sponges, were employed for stimulation, delivering a 2-mA current. The anode electrode was positioned over the primary motor cortex, corresponding to C3/C4 electrode sites, while the cathode electrode was situated over the contralateral supraorbital region (Nasseri et al. 2015). All electrodes were secured in place with soft elastic straps.

Following the 20-minute tDCS session, patients underwent a 20-minute CIP session, which included 5 minutes of infra-red (IRR) therapy to the affected upper and lower limbs, followed by 15 minutes of neck flexion with rotation, neck extension with rotation, rhythmic stabilisation, alternating isometrics of the trunk, and various contractions targeting pelvis and scapula positioning.

Patients were scheduled for weekly physiotherapy/tDCS appointments, conducted three times per week over a period of four weeks (one month). Post-intervention assessment was performed three days after the final treatment session.

## Task-Specific Exercise (TSE) Intervention

The TSE programs were designed based on principles from movement science and motor learning literature, emphasising goal-directed practice and repetition (Arabzadeh et al. 2018; Chaturvedi et al. 2018). Pre-intervention assessments of baseline parameters were conducted three

days before the exercise regimen. Each TSE session lasted between 20 to 30 minutes, during which patients' functional deficits were evaluated, and detailed explanations were provided to both patients and their caretakers regarding the procedure plan.

The exercise protocol comprised ten sets of programs involving both upper and lower extremities, categorised into sets A and B. Set A exercises included six sets of tasks such as seated reaching, stepping exercises in various directions, knee flexion and extension, stepping over obstacles, and sit-to-stand transitions with walking. Set B exercises incorporated somatosensory and visual manipulations, including exercises performed with open or closed eyes on hard or soft surfaces. These exercises included sitting on a Swiss ball, double-leg standing, tandem standing, and tandem walking, with variations in visual input as described. Additionally, patients received 5 minutes of IRR therapy to the affected upper and lower limbs, followed by 15 minutes of PNF exercises targeting the neck, trunk, and affected upper and lower limbs, as part of the CIP regimen. Patients attended three weekly sessions over the course of one month. Post-intervention assessment was conducted three days after the final session.

## Control Intervention Physiotherapy (CIP)

Each session of CIP spanned 20 minutes. Initial baseline assessments of the primary outcome measure, BDNF and socio-demographic characteristics of the patients were conducted three days before commencing PNF exercises. Patients' functional deficits were evaluated, and detailed explanations regarding the exercise regimen were provided to both patients and caretakers.

During the CIP session, patients received a 5-minute IRR session followed by 15 minutes of PNF exercises in a cephalo-caudal direction. These exercises included neck flexion with rotation, neck extension with rotation, rhythmic stabilisation, alternating isometrics of the trunk, and various contractions to address pelvis and scapula positioning. Patients underwent the designated PNF exercises three times per week. Subsequently, post-intervention assessments were conducted accordingly.

## Combined Interventions

Each treatment session for patients in this group lasted 20 minutes and comprised 20 minutes of tDCS, 20 to 30 minutes of TSE, and an additional 20 minutes of CIP. Pre-intervention assessments, conducted three days before the commencement of treatment, included evaluating serum BDNF levels and other relevant variables. Additionally, patients' clinical deficits were assessed, informed consent was obtained, and explanations regarding the nature and plan of the treatment regimen were provided to both patients and their caregivers.

During each session, patients underwent 20 minutes of tDCS using

an International 10-20% EEG montage. Subsequently, they engaged in 20 to 30 minutes of TSE exercises targeting the upper and lower limbs, with five 5-minute rest periods following the initial tDCS intervention. Following the TSE exercises, patients assumed a supine position and received 5 minutes of IRR followed by 15 minutes of PNF as part of the CIP regimen.

Patients attended clinical visitations three times per week over a period of four weeks. Post-intervention assessments were conducted three days after the final session.

### Statistical analysis

Data analysis was conducted using IBM SPSS 23.0 software. Descriptive statistics, including frequency, mean, standard deviation (SD), and percentages, were used to analyse demographic characteristics. The Shapiro-Wilk test was employed to assess the normality of continuous variables. One-way ANOVA was conducted to determine differences in serum BDNF levels among the groups, and Bonferroni post hoc tests were used to identify specific differences between treatment groups. A significance level of  = 0.05 was set for all statistical analyses.

## RESULTS

The age distribution in each group mainly ranged from 35 to 49 and 50 to 65 years, with the latter age group being predominant. Patients in the TSE and tDCS groups showed an age distribution of 73.3% and 80% between 50-65 years, while those in the control group had 86.7% within the same age range. In contrast, the combined TSE and tDCS group exhibited a distribution of 13.3% and 86.7% between 35-49 and 50-65 years, respectively. The gender distribution showed that a significant majority of patients were male, with the highest proportion of 73.3% seen in the tDCS group, in comparison to 60%, 53.3%, and 66.7% in the TSE, combined TSE + tDCS, and control groups, respectively.

Most patients in each group were married and resided in rural areas, with a small number being either divorced or widowed. Analysis of the patients' BMI revealed that 53.3% of individuals in the tDCS and control groups had a normal BMI, compared to 46.7% in the combined TSE + tDCS intervention group. Additionally, 53.3% and 40% of patients in the combined TSE + tDCS and TSE groups were classified as pre-obese. Most patients exhibited normal systolic (SBP) and diastolic (DBP) blood pressure, though increased SBP was observed in 53.3% and 26.7% of patients in the tDCS and combined TSE + tDCS group. Table 1 indicated no significant differences in baseline characteristics among the groups.

A statistically significant difference was observed in the effect of interventions on the serum BDNF levels of stroke patients (F=58.04, P=0.001). The baseline concentrations of serum BDNF for the control group, TSE + CIP, tDCS + CIP, and combined TSE + tDCS + CIP were 120.08 pg/ml, 117.07 pg/ml, 116.01 pg/ml, and 116.81 pg/ml, respectively. Notably, patients

TABLE 1: Frequency distribution of the sociodemographic characteristics of the stroke patients (n = 60)

| Characteristics | Interventions Groups | | | | p-value |
|---|---|---|---|---|---|
| | TSE n(%) | tDCS n(%) | TSE + tDCS n(%) | Control n(%) | |
| Gender | | | | | 0.952 |
| Male | 9 (60.0) | 11 (73.3) | 8 (53.3) | 10 (66.7) | |
| Female | 6 (40.0) | 4 (26.3) | 7 (46.7) | 5 (33.7) | |
| Age (years) | | | | | 0.517 |
| 20-34 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| 35-49 | 4 (26.7) | 3 (20.0) | 2 (13.3) | 2 (13.3) | |
| 50-65 | 11 (73.3) | 12 (80.0) | 13 (86.7) | 13 (86.7) | |
| Marital status | | | | | 0.270 |
| Single | 0 (0) | 0 (0) | 0 (0) | 0 (0) | |
| Married | 12 (80.0) | 9 (60.0) | 8 (53.3) | 12 (80.0) | |
| Separated | 3 (20.0) | 4 (33.3) | 5 (33.3) | 2 (13.3) | |
| Widow | 0 (0) | 1 (6.7) | 2 (13.3) | 1 (6.7) | |
| Mean ± SD | 56.13 ± 6.50 | 56.7 ± 6.19 | 57.47 ± 5.55 | 56.87 ± 4.97 | |
| Residence | | | | | 0.922 |
| Rural | 11 (73.3) | 8 (53.3) | 9 (60.0) | 9 (60.0) | |
| Urban | 4 (26.7) | 7 (46.7) | 6 (40.0) | 6 (40.0) | |
| Occupation | | | | | 0.089 |
| Civil servants | 2 (13.3) | 3 (20.0) | 3 (20.0) | 4 (26.7) | |
| Skilled laborers | 2 (13.3) | 2 (13.3) | 0 (0) | 0 (0) | |
| Unemployed | 4 (26.7) | 0 (0) | 3 (20.0) | 3 (20.0) | |
| Small traders | 7 (46.7) | 10 (66.7) | 9 (60.0) | 8 (53.3) | |
| Retired | 0 (0) | 0 (0) | 0 (0) | 0 (0) | |
| Education | | | | | 0.393 |
| None | 0 (0) | 0 (0) | 0 (0) | 0 (0) | |
| Non-formal | 1 (6.7) | 2 (13.3) | 3 (20.0) | 3 (20.0) | |
| Primary | 6 (40.0) | 2 (13.3) | 4 (26.7) | 3 (20.0) | |
| Secondary | 5 (33.3) | 7 (46.7) | 4 (26.7) | 5 (33.3) | |
| Post-Secondary | 3 (20.0) | 4 (26.7) | 4 (26.7) | 4 (26.7) | |
| Stroke laterality | | | | | 0.858 |
| Right | 10 (66.7) | 13 (86.7) | 11 (73.3) | 12 (80.0) | |
| Left | 5 (33.3) | 2 (13.3) | 4 (26.7) | 3 (20.0) | |
| SBP (mmHg) | | | | | 0.520 |
| <140mmHg | 9 (60.0) | 7 (46.7) | 11 (73.3) | 10 (66.7) | |
| ≥140mmHg | 6 (40.0) | 8 (53.3) | 4 (26.7) | 5 (33.3) | |
| Mean ± SD | 135.53 ± 8.12 | 136.53 ± 11.53 | 132.13 ± 9.59 | 135.40 ± 5.61 | |
| DBP (mmHg) | | | | | 0.634 |
| <90mmHg | 14 (93.3) | 14 (93.3) | 11 (73.3) | 12 (80) | |
| >90mmHg | 1 (6.7) | 1 (6.7) | 4 (26.7) | 3 (20) | |
| Mean ± SD | 76.20 ± 9.81 | 76.80 ± 9.07 | 81.13 ± 8.67 | 76.87 ± 11.19 | |
| BMI (kg/m$^2$) | | | | | 0.570 |
| Underweight | 0 (0) | 0 (0) | 0 (0) | 0 (0) | |
| Normal | 9 (60.0) | 8 (53.3) | 7 (46.7) | 8 (53.3) | |
| Overweight | 6 (40.0) | 7 (46.7) | 8 (53.3) | 7 (46.7) | |
| Obesity | 0 (0) | 0 (0) | 0 (0) | 0 (0) | |
| Mean ± SD | 24.23 ± 2.57 | 24.23 ± 2.42 | 24.27 ± 2.55 | 25.11 ± 2.31 | |
| Mini-Mental State Examination (MMSE) | | | | | 0.432 |
| Mean ± SD | 23.4 ± 1.50 | 23.6 ± 1.29 | 23.3 ± 1.28 | 24.3 ± 1.09 | |

| | | | | | |
|---|---|---|---|---|---|
| Baseline Serum BDNF | | | | | 0.345 |
| Mean ± SD | 118.4 ± 11.51 | 117.8 ± 7.57 | 119.7 ± 11.91 | 121.1 ± 14.12 | |

BDNF: brain-derived neurotrophic factor; BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure; SD: standard deviation; tDCS: transcranial direct current stimulation; TSE: task-specific exercises

who received combined interventions exhibited the highest median post-test serum BDNF concentration (194.57 pg/ml) compared to the other groups. These findings indicated a positive effect of the interventions on serum BDNF levels, with a marked increase observed in patients receiving tDCS and combined TSE + tDCS + CIP interventions (Figure 2).

After the 4-week intervention of TSE, the serum BDNF levels were 41.67pg/ml lower than those who received tDCS, and 61.75pg/ml lower than those who received the combined TSE + tDCS intervention. Additionally, patients in the combined TSE + tDCS and tDCS groups had higher serum BDNF levels, with an increase of 69.96pg/ml and 49.89pg/ml, respectively, compared to those in the control group. No significant difference was observed in serum BDNF levels between those who received TSE and the control group. Patients who received tDCS intervention had serum BDNF levels lower by 20.08pg/ml compared to those who received the combined TSE + tDCS intervention (Figure 3).

## DISCUSSION

Stroke survivors experience a wide range of clinical symptoms, including motor control deficits, coordination impairments, neuropsychological

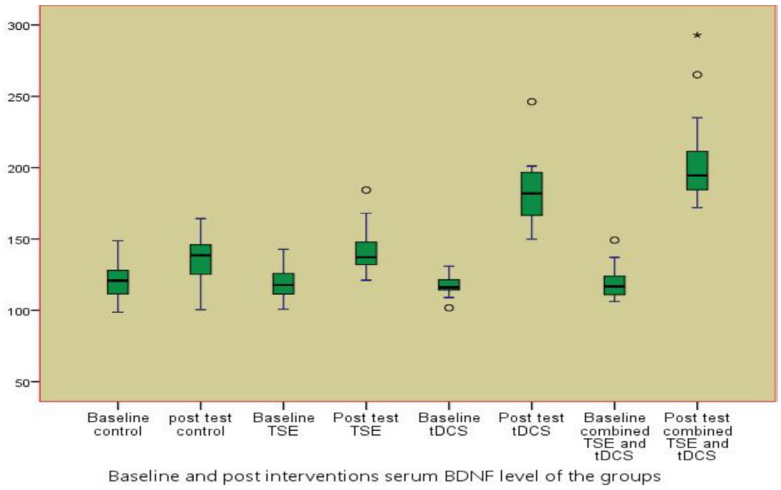


FIGURE 2: The effect of 4-week interventions on the patients' serum brain-derived neurotrophic factor (BDNF) levels. tDCS: transcranial direct current stimulation, TSE: task-specific exercises

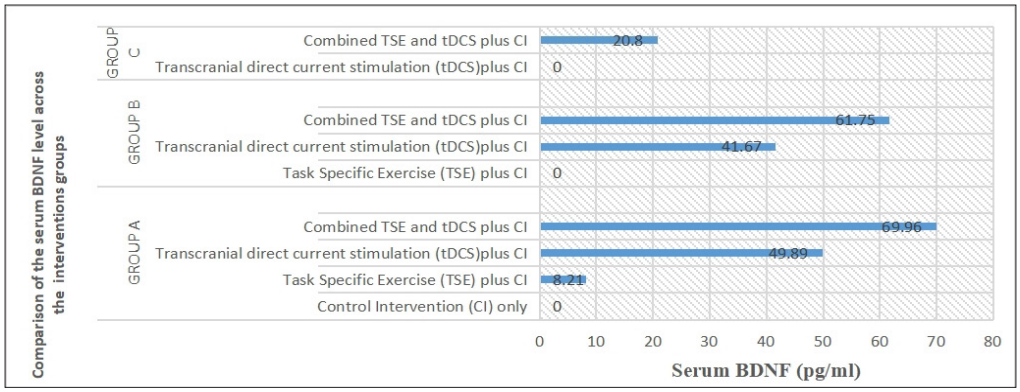


FIGURE 3: Post hoc comparison of the studied interventions. BDNF: brain-derived neurotrophic factor, CI: control intervention physiotherapy, tDCS: transcranial direct current stimulation, TSE: task-specific exercises

issues, and gait disturbances. To address these symptoms and improve functional outcomes, physical therapy interventions aim to stimulate various neuronal growth proteins that promote neural and synaptic plasticity, preventing the recurrence of stroke and its complications. Among these growth proteins, increased BDNF expression and its functions through the tropomyosin receptor kinase B (TrkB) pathway play a critical role in the central nervous system (Begliuomini et al. 2008). TrkB receptors act in various brain regions, such as the hippocampus, frontal cortex, visual cortex, superior colliculus and cerebellum, promoting synaptic and neuroplasticity in neurodegenerative diseases and stroke (Mateen & Munawar 2020; Yoo & Kim 2015). A genetic variation in the BDNF gene, the substitution of valine with methionine at codon 66 (Val66Met), is associated with lower serum BDNF levels (Cain et al. 2017).

Low serum BDNF levels are significantly related to poor functional outcomes, higher mortality rates, increased chances of recurrence, and diminished synaptic and neuroplasticity in stroke patients. Research reports indicate that changes in synaptic excitability are relevant to stroke rehabilitation (Teskey et al. 2022). Recovery from stroke episodes is correlated with plastic changes in cerebral organisation (Murphy & Corbett 2009). Dynamic neuroplastic processes are initiated in stroke survivors, involving increased perilesional excitability mediated by excitatory neurotransmitters in the acute and subacute phases. In the chronic phase, more complex modifications of intracortical and interhemispheric inhibition are involved (Schlaug & Renga 2008). Post-stroke production and release of neural growth factors, including BDNF, generate a favourable environment for neuronal regeneration in the perilesional cortex, facilitating stroke recovery (Murphy & Corbett 2009).

In the present pilot study, patients

who received tDCS interventions demonstrated increased serum BDNF levels compared to those in the TSE and control groups. Brain stimulation techniques, such as tDCS, have demonstrated the ability to induce neuronal plasticity, synaptic changes and enduring alterations in cortical excitability (Fritsch et al. 2010). Specifically, tDCS has been associated with increased BDNF release and cortical reorganisation, yielding cognitive benefits for stroke patients (Guo et al. 2020; Lee et al. 2018). Anodal tDCS, as utilised in this pilot study, has shown efficacy in enhancing hippocampal function, reducing levels of proinflammatory cytokines (IL-1β and TNFα), and augmenting BDNF expression and release (Lee et al. 2018). Previous research indicates that anodal tDCS induces enduring, polarity-dependent modifications in neocortical excitability and reinforces synaptic connectivity. This effect triggers the release of neurotrophic factors including BDNF, by modulating brain membrane function through depolarisation-induced events, leading to increased intracellular calcium ($Ca^{2+}$) levels via N-methyl-D-aspartate receptor (NMDAR) and voltage-gated calcium channel activation (Cocco et al. 2018). Elevated $Ca^{2+}$ levels facilitate the activation and acetylation of the transcription factor cAMP response-element-binding protein (CREB/CBP), as well as increased noradrenaline release by cortical astrocytes and epigenetic modifications (Monai & Hirase 2016; Monai et al. 2016).

Furthermore, it is believed that anodal tDCS enhances BDNF exon I and IX mRNA, and protein expression by promoting CREB phosphorylation at Ser133. It also increases binding of activated CREB to BDNF promoter I, thus recruiting the transcriptional coactivator CBP to BDNF promoter I, then induces histone acetylation and epigenetic modifications at BDNF promoter I, including increased acetylation of histone 3 at lysine 9 (H3K9) (Cocco et al. 2018; Podda et al. 2016). This mechanism is thought to depend on CREB activation, CBP recruitment, and H3K9 acetylation. Animal studies have reported that CRE mutation contributes to activity-mediated induction of rat BDNF promoter I, contrasting the differential regulation of promoter I activity by CREB transcription observed in humans and rodents (Cortés-Mendoza et al. 2013).

TSE has demonstrated a correlation with enhanced cortical reorganisation. Research indicates that TSE can facilitate functional restoration by engaging non-affected brain regions adjacent to the lesion and supplementary brain areas (Plautz et al. 2000). Physical activity, such as TSE, triggers the coactivation of mitochondrial biogenesis pathways, including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and oestrogen-related receptor alpha (ERRα), consequently stimulating the release of insulin-like growth factor-1 (IGF-1) from the liver. IGF-1, in turn, promotes BDNF gene expression through FNDC5-mediated irisin production, which can induce further BDNF gene expression in the hippocampus (Phillips et al. 2014). Moreover,

exercise exerts a direct influence on neurotransmission by activating dopaminergic, noradrenergic, and serotonergic systems. Peripheral release of catecholamines triggers the activation of the calcium-calmodulin system, leading to elevated serum calcium ion levels, which serve as essential ion transporters and transcription factors like CREB, pivotal for long-term neuronal plasticity and BDNF augmentation (Di Liegro et al. 2019; Ding et al. 2006).

The present study findings revealed that following a four-week TSE intervention, there was a notable rise in serum BDNF levels, linked to increased intracellular calcium ion influx and CREB binding. However, this elevation did not significantly differ from the control group, potentially due to factors such as sample size and the short intervention period. In contrast, stroke patients receiving combined TSE and tDCS interventions in our pilot study actively engaged in repeated, meaningful tasks involving their affected upper and lower limbs. This approach aligns with recommendations from various research studies advocating for increased, repetitive, and meaningful task-oriented rehabilitation (French et al. 2008). Notably, the combined TSE and tDCS interventions proved significantly more effective in elevating serum BDNF levels compared to TSE, tDCS, and conventional physiotherapy treatments administered individually.

The pilot study was conducted over a four-week treatment period. Future research with longer treatment durations, follow-up assessments to gauge the retention effect, and larger sample sizes should be undertaken. Additionally, future studies should aim to identify stroke patients in the acute, sub-acute and chronic stages who may benefit more from the respective interventions. Investigating patients' characteristics and their correlation with interventions will help to identify causative factors for treatment outcomes. Finally, future studies should systematically report adverse effects of the treatments to provide a comprehensive view of their safety and efficacy.

## CONCLUSION

In lieu of a conclusion, the present pilot study underscores the importance of serum BDNF in post-stroke recovery. TDCS showed promise in boosting BDNF release, while TSE demonstrated potential, though not statistically significant within our four-week study. The combined TSE and tDCS group exhibited the most substantial increase in serum BDNF, suggesting a synergistic effect. Engaging in meaningful tasks and repeated stimuli may enhance BDNF expression and the recovery process. These findings provide valuable insights into interventions, through BDNF modulation, for improving post-stroke outcomes. The practical implication is that a combined regimen of TSEs and transcranial stimulation holds promise for enhancing post-stroke recovery, warranting further investigation in larger, more comprehensive studies.

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