ORIGINAL ARTICLE

Evaluation of Serum Brain-derived Neurotrophic Factor (BDNF) in Ambulatory Stroke Survivors With Mild Cognitive Impairment and Normal Cognitive Functions

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ABSTRACT

Introduction: This study aims to assess serum brain-derived neurotrophic factor levels in ambulating stroke patients, focusing on potential correlations with cardiovascular risk factors and overall obesity. **Materials and methods:** Sixty stroke patients (63.3% male, 36.7% female, mean age: 57 years) participated. Quantikine ELISA kit (SL0371Hu) was used to determine post-stroke serum brain-derived neurotrophic factor levels. Patient demographics, including body mass index, systolic, and diastolic blood pressures, were recorded and computed to assess patients' overall obesity status and blood pressure. **Results:** The mean serum brain-derived neurotrophic factor level was 119.25 (SD = 11.40) pg/ml. No significant effect of selected anthropometric indices and cardiovascular risk factors, including age (p = 0.493), overall obesity status (p = 0.848), systolic and diastolic blood pressures (p = 0.840 and 0.540, respectively), on the serum brain-derived neurotrophic factor. **Conclusion:** In ambulating stroke patients, the serum brain-derived neurotrophic factor levels were within the normal range but tended towards the lower limit. Consequently, it is crucial to develop and implement medical and rehabilitation programs specifically aimed at increasing serum brain-derived neurotrophic factor levels in post-stroke patients.
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Keywords: Age-related decline, Cardiovascular risk, Neurotrophic factor, Obesity, Rehabilitation

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INTRODUCTION

Stroke, marked by a sudden loss of body function due to interrupted blood flow to the brain (1,2), stands as the second leading cause of global morbidity, mortality, and disability, with its incidence doubling after the age of 55 years (3). The abrupt onset of a stroke leaves individuals and their families ill-prepared to cope with its consequences (4). A significant percentage of stroke survivors (25%-50%) receive care at home from family members, intensifying the burden and sustaining stress for both patients and caregivers (5).

Stress and psychological diseases are often perceived as individual processes influenced by environmental, psychological, or biological factors (6,7). However, post-stroke neuropsychological symptoms, including stress, depression, and anxiety, can inflict substantial damage on various organs and systems, particularly the brain. The impact on the central nervous system (CNS) may contribute to the development of cardiovascular diseases (CVD) and mental disorders, such as anxiety, cognitive decline, and depression, significantly affecting the lives of both patients and their caregivers (8). Loss and impairment of walking ability are among the most devastating outcomes of post-stroke hemiplegia. Approximately one-third of stroke survivors, particularly those in the acute stage, are never able to walk independently. In contrast, ambulating stroke survivors, who can walk with or without assistance, are typically in the sub-acute or chronic stages of stroke recovery. However, even among these ambulatory patients, walking impairments persist, with 70% still experiencing difficulties in community ambulation (9).

Brain-derived neurotrophic factor (BDNF), a well-known neurotrophic factor expressed broadly in various brain

regions (10,11,12,13,14,15), emerges as a potential key player (12,16,17,18). It is secreted by neurons and can traverse the blood-brain barrier (11). Prior studies have highlighted the positive correlation between BDNF levels in the CNS and the peripheral system, linking CNS BDNF to the development of mental disorders and reduced cognition (19,20). This relationship suggests considering peripheral BDNF as a potential marker of mental diseases in humans (16,17,20,21).

BDNF plays a crucial role in maintaining neuronal survival, regulating neurogenesis, and determining the functional architecture of neurons in the adult brain (12,18). It is essential in cell differentiation, migration, dendritic arborization, synaptogenesis, and synaptic plasticity (18). Following damage to a developing and mature brain, BDNF is instrumental in restoration and resistance to damage (18). Serum BDNF levels have been associated with clinical diseases, including Alzheimer's, dementia, diabetes, depression, and acute coronary syndrome (22). Stress has been shown to influence the expression of BDNF in critical brain regions, further linking psychological disorders to the intricate BDNF system (7,8,14,21,23).

Clinical studies have revealed a significant interaction between the BDNF gene and the serotonin transporter gene, conveying vulnerability to depression, particularly when exacerbated by stressful life events (18). The decline in BDNF levels was also observed to be associated with impaired cognitive function and post-stroke depression (PSD) development in stroke patients (24,25). The levels of circulating BDNF decrease during the acute phase of ischemic stroke, and diminished concentrations are linked to unfavourable long-term functional outcomes (26).

The study aims to address the current research gap by evaluating serum BDNF levels at different stages of stroke, specifically focusing on mild cognitive impairment and normal cognitive function in ambulatory stroke survivors.

MATERIALS AND METHODS

This cross-sectional study involved the recruitment of 67 ambulating stroke patients, with seven exclusions due to non-compliance with inclusion criteria (Figure 1). Inclusion criteria encompassed participants aged between 35 and 65 years with no history of drug abuse, absence of psychiatric diseases, controlled systemic hypertension, non-use of antidepressants, and antihypertensive or anti-diabetic drugs. Participants were required to score 20 and above in the mini-mental state examination (MMSE) indicating that they have mild impaired cognition or normal cognitive function, exhibit no visual or auditory deficits, refrain from participation in other research studies, ambulate freely or with mild assistance, sustain a stroke for six months or more, and respond to verbal commands. Exclusions pertained to those with moderate to severe musculoskeletal disorders in the upper and lower extremities, and those with balance impairments.

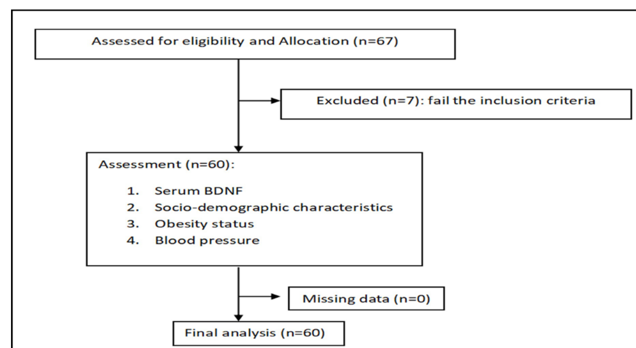

Figure 1: Flow chart of the study.

Cognitive function assessment utilized the MMSE, with scores ranging from 0 to 30. Mild cognitive impairment and normal cognitive performance were indicated by scores between 20 and 23, and >24, respectively (27). Serum BDNF levels were quantified using the ELISA method with Sunlung biotech Human BDNF ELISA Kit reagents (SL0371Hu). The research was carried out at Imamu Wali General Hospital in Kano, Nigeria, following approval from the Health Research Ethics Committee of the Ministry of Health, Kano State, Nigeria (NHREC/17/03/2018), ensuring compliance with ethical standards throughout the study. The approval from the health research ethics committee was granted on April 12, 2023.

Anthropometric Measurement

Anthropometric measurements focused on body mass index (BMI), calculated using the standard formula body weight (in kilogram) divided by height (in meter square). Participants' weight and height were measured using Seca© weight and height scales, ensuring precision in in kilograms (kg) to the nearest 0.1 kg, and centimetres (cm) to the nearest 0.1cm respectively. During the measurements, participants wore only light clothing to minimize any potential interference with the readings. Shoes were removed before weighing to capture an accurate representation of the participant's body mass. For height measurement, participants were instructed to stand with heels close together, ensuring a consistent and stable stance. They were asked to look ahead horizontally to maintain a standardized posture throughout the measurement process. These meticulous procedures were followed to obtain reliable anthropometric data, crucial for the comprehensive assessment of the participants' physical characteristics. To maintain accuracy, both weight and height were measured thrice. These standardized procedures ensured

precise and consistent anthropometric measurements across all study participants (28).

Blood Pressure (BP) Measurement

Validated, standardized, and calibrated digital and manual sphygmomanometers, equipped with appropriate cuff sizes, were employed to measure the blood pressure (BP) of the patients. The BP readings were obtained through both automated and manual methods, adhering to the manufacturer's operating instructions. Three measurements were taken, and the average of these readings was recorded as the individual systolic (SBP) and diastolic blood pressure (DBP). During the procedure, the inflatable bladder cuff was wrapped around at least 80% of the arm, ensuring optimal coverage, 2cm above the brachial artery. In the manual method, an appropriately sized arm cuff was carefully selected and placed 2cm above the brachial artery on the participant's arm. After inflation of the arm cuff, the diaphragm of the stethoscope was positioned over the patient's brachial artery. The cuff was then slowly deflated at an approximate rate of 2 to 3mm per second, with careful attention paid to the appearance of the first Korotkoff sound (indicating SBP) and the final disappearance of all sound (fifth phase of Korotkoff), indicating DBP (29). This meticulous approach ensured accurate and reliable blood pressure measurements across the participant cohort.

Serum BDNF Analysis

Blood samples were obtained in the morning, between 8:00 and 9:00 AM to minimize circadian effects. The serum extraction and analysis were conducted at Imamu Wali General Hospital and Plasma Diagnostics Limited, Kano State, Nigeria. The Microelisa strip plate provided in the kit was pre-coated with a BDNF-specific antibody. Standards or samples were introduced into the designated wells of the Microelisa strip plate, binding to the specific antibody. Subsequently, a Horseradish Peroxidase (HRP)-conjugated antibody, specific to BDNF, was added to each well and incubated. After incubation, free components were thoroughly washed away. To visualize the presence of BDNF, 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution was added to each well. Only those wells containing BDNF and the HRP-conjugated BDNF antibody exhibited a blue colour, transitioning to yellow upon the addition of the stop solution. The optical density (OD) was measured using a spectrophotometer at a wavelength of 450 nm. The OD values were directly proportional to the concentration of BDNF, providing a reliable quantitative measure for the serum analysis. This meticulous process ensured the accuracy and consistency of BDNF concentration assessment in the collected blood samples.

Statistical Analysis

The data were analysed using IBM Statistical Package for the Social Sciences (SPSS) version 23.0, with a predetermined significance level set at 0.05. Descriptive statistics including frequency, mean, standard deviation, and percentage were employed to analyse the socio-demographic characteristics of the participants. Factorial ANOVA was used to determine the effect of selected cardiovascular risk factors on serum BDNF levels in stroke patients. This statistical test allowed for a rigorous examination of the differences in BDNF concentrations between groups defined by cardiovascular risk factors. The results of this analysis provided valuable insights into the potential influence of these factors on the serum BDNF levels of the stroke patient cohort.

RESULTS

The study included 60 participants with a mean age of 56.78 (SD = 5.70) years, predominantly within the 50-65 age range (81.7%), while those aged 35-49 constituted 18.3%. Females constituted 63.3% of the entire cohort. A majority resided in rural areas (66.1%), and the majority were married (68.3%), with a smaller proportion being separated (25%) or widowed (6.7%). All participants, displaying mild to moderate residual deformity, were ambulatory and in the recovery stage. The prevalence of right hemiplegic gait was higher (76.7%) than left hemiplegic gait (23.3%). Educationally, participants covered a spectrum: 15% non-formal, 25% primary, 35% secondary, and 25% post-secondary education. Employment distribution included 16.7% unemployed, 56.7% engaged in small businesses, 20% as civil servants, and 6.7% as skilled labourers.

Approximately 45% of participants were at risk of overall obesity, posing potential cardiovascular risks. Most participants exhibited normal blood pressure, with 38.3% and 15% showing elevated SBP and DBP, respectively. The mean serum BDNF level was 119.25 (SD = 11.40) pg/ml, and the mean MMSE score was 23.38 (SD = 1.28). These findings provide a comprehensive overview of the demographic and clinical characteristics of the study participants, essential for understanding the context of subsequent analyses and interventions. Table I summarizes the characteristics of the whole cohort of participants.

Table I: Frequency distribution of the sociodemographic characteristics of the stroke patients (n = 60)

Characteristics	n (%)	Mean (SD)	p-value
Gender		1.37 (0.49)	0.952
Males	38 (63.3)		
Females	22 (36.7)		
Marital status		1.38 (0.61)	0.270
Single	0 (0.0)		
Married	41 (68.3)		
Separated	15 (25.0)		
Widow	4 (6.7)		
Residence		1.38 (0.49)	0.922
Rural	37 (61.7)		
Urban	23 (38.3)		

CONTINUE

Table I: Frequency distribution of the sociodemographic characteristics of the stroke patients (n = 60). (CONT.)

Characteristics	n (%)	Mean (SD)	p-value
Age (years)		56.78 (5.70)	0.517
35-49	11 (18.3)		
50-65	49 (81.7)		
Stroke presentation (laterality)		1.23 (0.43)	0.858
Right sided	46 (76.7)		
Left sided	14 (23.3)		
Occupation		3.83 (1.63)	0.089
Civil servant	12 (20.0)		
Skilled labourers	4 (6.7)		
Unemployed	10 (16.7)		
Small traders	34 (56.7)		
Retired	0 (0.0)		
Education level		3.70 (1.01)	0.393
Non-formal	9 (15.0)		
Primary	15 (25.0)		
Secondary	21 (35.0)		
Post-secondary	15 (25.0)		
None	0 (0.0)		
Systolic blood pressure (mmHg)		134.00 (8.89)	0.520
<140	37 (67.7)		
≥140	23 (38.3)		
Diastolic blood pressure (mmHg)		77.75 (9.70)	0.634
<90	51 (85)		
≥90	9 (15)		
Body mass index (BMI) (kg/m^2)		24.45 (2.43)	0.761
Underweight	0 (0)		
Normal	33 (55)		
Pre-obesity	27 (45)		
Obesity	0 (0)		
BDNF level (pg/mL)	-	119.25 (11.40)	0.100
Mini-mental state examination (MMSE)	-	23.38 (1.28)	-

BDNF = brain-derived neurotrophic factor; SD = standard deviation

The serum BDNF levels of the entire cohort of patients were within the normal range (28-1000 pg/ml) but tended towards the lower limit. No significant difference in serum BDNF levels across the studied cardiovascular risk factors were observed (Table II). However, participants at the pre-obesity stage (119.76 pg/ml) and those with normal DBP (118.67 pg/ml) demonstrated slightly higher serum BDNF levels compared to counterparts with normal BMI (118.84 pg/ml) and elevated DBP (112.53 pg/ml). The effect of the interaction of the variables on the serum BDNF levels did not reveal any statistically significant difference as shown in Table III.

Table II: Effect of selected cardiovascular risk factors on serum BDNF of ambulatory stroke patients

Variables	n (%)	Serum BDNF level (pg/mL) X ± SE	F	p-value
Age (years)			0.476	0.493
35-49	36 (60)	121.89 ± 4.06		
50-65	24 (40)	115.29 ± 2.14		
Body mass index (BMI) (kg/m^2)			0.041	0.848
18.5-24.9	33 (55)	118.84 ± 2.97		
25-29.9	27 (45)	119.76 ± 3.01		
Systolic blood pressure (mmHg)			0.041	0.840
<140	37 (61.7)	119.14 ± 2.58		
≥140	23 (39.3)	119.44 ± 2.58		
Diastolic blood pressure (mmHg)				
<90	51 (85)	118.67 ± 1.96	0.337	0.540
≥90	9 (15)	112.53 ± 4.30		

BDNF = brain-derived neurotrophic factor; SE = standard error

Table III: Interactions of the effect of selected cardiovascular risk factors on serum BDNF of ambulatory stroke patients

Variables	F	p-value
Age (years) * BMI (kg/m^2)	1.329	0.255
Age (years) * SBP (mmHg)	0.205	0.653
BMI (kg/m^2) * SBP (mmHg)	0.303	0.583
BMI (kg/m^2) * DBP (mmHg)	0.157	0.693
Age (years) * BMI (kg/m^2) * SBP (mmHg)	0.415	0.529
BMI (kg/m^2) * SBP (mmHg) * DBP (mmHg)	0.386	0.537

BDNF = brain-derived neurotrophic factor; BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure

DISCUSSION

The concept of neuroplasticity is pivotal in enhancing health and well-being, with impaired brain function often linked to neurotrophic factors, particularly serum BDNF. Elevated serum BDNF levels and other neurotrophic factors contribute to hippocampal and regional brain resilience (15,30), providing protection against cell death by promoting cell proliferation, maturation, and neurogenesis, and the growth and functioning of neurons, particularly in neurodegenerative disorders (31,32).

The synthesis of BDNF involves the precursor form proBDNF, which transforms into mature BDNF (mBDNF), a process detailed by Numakawa et al. (12) and Lu (33). BDNF interacts with receptors, tropomyosin receptor kinase B (TrkB) and p75 neurotrophin receptor (p75NTR), with distinct functions outlined by Teng et al.

(34) and Hwang et al. (35). Metal ions, like Cu2+ and Zn2+, activate BDNF signalling, converting proBDNF to mBDNF (34). ProBDNF, secreted in various conditions, binds to p75NTR, triggering apoptosis (35). In contrast, mBDNF binds to TrkB, promoting cell survival and spine complexity (36,37). Co-expression of p75NTR with TrkB regulates BDNF activity in non-pathological conditions (38). BDNF concentrations vary in brain regions (39,40), and lower levels are found in other organs such as liver, heart, and lungs (13). Pan et al. (11) and Klein et al. (19) discuss BDNF transport and signaling across the blood-brain barrier. These mechanisms are essential for interpreting serum BDNF levels and their potential impact on brain function. The impact of BDNF can be counteracted by neurodegenerative factors, including pro-inflammatory cytokines (16). Given the implication of BDNF in diverse clinical conditions, it becomes crucial to investigate its levels in individuals who have survived a stroke (19,40).

Neurological and psychiatric diseases, including stroke, often exhibit compromised BDNF integrity, worsening symptoms, and cognitive impairment (15,16,21,27,30). In alignment with findings by Aisyah et al. (41), this study revealed lower serum BDNF levels in stroke patients, especially those with cognitive impairment. Lower BDNF levels are associated with oxidative stress and white matter regional impediments, particularly in late-onset bipolar disorder and psychological symptoms. This emphasizes the intricate relationship between BDNF, stressors, and cognitive outcomes (31,32). Stressors elevate BDNF immune-reactive pyramidal neurons, associated with hypothalamus-pituitary-adrenal axis up-regulation and short-term memory processing during stress (14).

Psychological disorders, potential stroke risk factors, impact brain function through two systems: the stress-related system (hypothalamus-pituitary-adrenocortical axis and hippocampus) and the reward-associated system (ventral tegmental area (VTA) and nucleus accumbens (NAc) pathway) (21). The Val66Met polymorphism in the BDNF gene contributes to impaired cellular processing, geriatric depression, and altered BDNF secretion (18). This genetic variation is associated with poorer episodic memory and abnormal hippocampal activation (18). BDNF, a key neurotrophin in neurogenesis and neuronal function, has implications for cognitive outcomes in stroke survivors (12,33). Stress hormone effects are central, linking stress to altered BDNF levels and suggesting stress's potential influence on cognitive function in stroke survivors (8,10,21).

As the population of CVD patients increases, it becomes crucial to explore factors associated with adverse outcomes in CVD. potentially revealing novel therapeutic strategies and treatment plans. Chronic mental stress has been linked to the development and progression of stroke, emphasizing the need for a comprehensive understanding of the interplay between neurotrophic factors, neurodegenerative elements, and stressors in CVD. The impact of BDNF can be counteracted by neurodegenerative factors, including pro-inflammatory cytokines. The American Heart Association's statistics underscore the importance of understanding cardiovascular health in stroke survivors (2). Our study did not find significant associations between BDNF and traditional cardiovascular risk factors.

Obesity, defined by an increase in the body's adipose tissue due to an imbalance between energy intake and expenditure, is associated with numerous health complications and can exacerbate comorbid conditions (42). It contributes to prolonged low-grade inflammation, with extensive evidence indicating that immune-mediated tissue inflammation is a critical factor linking obesity to insulin resistance (42). Several studies have identified an inverse relationship between plasma BDNF levels and BMI (43-47), suggesting that higher amounts of adipose tissue, which contain abundant natriuretic peptide clearance receptors, result in lower BDNF levels in obese individuals (42). Additionally, recent research has shown that peripheral elimination of BDNF is decreased in obese individuals (48).

However, our study found no significant effect of BMI on serum BDNF levels in stroke patients. Most participants had normal to pre-obesity BMI values, which may explain the lack of a direct effect on serum BDNF, alongside the small sample size. Furthermore, while previous research has indicated that serum BDNF concentration can vary widely in patients with systolic heart failure, with lower levels associated with severe heart failure (49), our study did not find a statistically significant effect of blood pressure on serum BDNF levels. This discrepancy may be due to differences in the timing of measurements and the sample size used in our study. These findings highlight the complex interactions between obesity, cardiovascular health, and BDNF levels, underscoring the need for targeted interventions to enhance BDNF levels in stroke patients.

CONCLUSION

Factors such as BMI, stroke presentation, and blood pressure did not significantly impact BDNF levels, suggesting the need for targeted interventions to enhance BDNF post-stroke, given its crucial role in cognitive function and neuroplasticity. While cardiovascular risk factors showed minimal influence on serum BDNF, these findings open avenues for further research. Future studies should involve larger sample sizes, additional cardiovascular and metabolic risk factors, and evaluate the effects of varied rehabilitation interventions on

serum BDNF. Additionally, investigating BDNF's impact on specific brain regions affected by stroke could yield valuable insights for improving recovery and cognitive outcomes in stroke survivors.

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