Original Article

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Quick Response Code:

Website: http://www.braincirculation.org

DOI: 10.4103/bc.bc_23_20

Brain-derived neurotrophic factor levels in acute stroke and its clinical implications

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Abstract:

BACKGROUND: Brain-derived neurotrophic factor (BDNF) has a very important role in repairing intact and injured brain, also known as neuroplasticity. Risk factors may affect neuroplasticity.

OBJECTIVES: In this study, our aim was to delineate the levels of BDNF in acute stroke with different etiology and impact of risk factors on its levels.

METHODS: In this prospective study, 208 patients with first-ever stroke, between 18 and 75 years, were included. All individuals were assessed for severity and type of stroke, risk factors, levels of BDNF in the acute stroke, and its association with outcome of stroke.

RESULTS: The mean age of the patients in our study was 55.29 ± 11.6 years. Compared to healthy controls, a significant decline in the levels of BDNF was observed after stroke ($P < 0.01$). Patients with National Institutes of Health Stroke Scale (NIHSS) <6 on the 1st day of stroke had significantly higher levels of BDNF than those with NIHSS >6 (9.8 ng/ml ± 3.8; $P < 0.01$). A significant difference in the levels of BDNF was observed on comparing the stroke patients and healthy individuals of age <55 and >55 years (<55 years: 10.4 ng/ml ± 3.2; >55 years: 9.8 ng/ml ± 4.5 and in healthy individuals <55 years: 22.97 ± 3.8, >55 years: 15.4 ± 4.9; $P < 0.01$). Risk factors have negative impact on levels of BDNF (diabetics, $P = 0.001$; alcoholics, $P = 0.003$; both diabetes mellitus + hypertension, $P = 0.002$; smokers, $P = 0.001$). The difference was not significant between hypertensives and nonhypertensives ($P = 0.06$).

CONCLUSION: BDNF level is significantly reduced in acute stroke. The presence of risk factors further affects its level.

Keywords:

Acute stroke, brain-derived neurotrophic factor, functional outcome, risk factors

Introduction

Brain-derived neurotrophic factor (BDNF) plays an important role in brain plasticity. Several studies have demonstrated the role of BDNF following stroke as a prognostic biomarker. BDNF is a member of the neurotrophin family that supports the survival of neurons in the nervous system by differentiation[1] and maturation[2] and shows a neuroprotective effect under adverse conditions, such as neurotoxicity, cerebral ischemia, and hypoglycemia. BDNF stimulates and controls the growth of new neurons from neural stem cells (neurogenesis). The high-affinity receptor for BDNF is tropomyosin receptor kinase B (TrkB). Neurotrophin signaling regulates the survival of cells by proliferation and growth of axon and dendrite through TrkB receptors. This neurotrophin (BDNF) is distributed throughout the healthy human brain.[2] Its action is related to the induction of antiapoptotic mechanisms and reducing the size of the lesion.[1]

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Submission: 06-07-2020
Revised: 29-07-2020
Accepted: 14-08-2020
Published: 30-09-2020

How to cite this article: Chaturvedi P, Singh AK, Tiwari V, Thacker AK. Brain-derived neurotrophic factor levels in acute stroke and its clinical implications. Brain Circ 2020;6:185-90.

Poststroke motor learning has been related to increases in BDNF concentrations in the cortex, cortical map reorganization through synaptogenesis, enhanced dendritic spine formation, and ramification, hence contributing in many ways to poststroke neuronal plasticity.[1] Despite the central role of BDNF in stroke recovery, the proper effect of stroke on cerebral BDNF production has been surprisingly poorly investigated. The aim of this prospective study was to evaluate the blood concentration of BDNF during the acute stage of first-ever stroke. Furthermore, we tried to find a potential association between serum levels of BDNF, severity of stroke, functional status, and various comorbidities in patients with stroke.

Methods

The prospective cohort study (in the period from November 2014 to April 2018) involved 208 patients of stroke aged 18–75 years with acute stroke. The diagnosis of stroke was based on neuroimaging procedures (computed tomography and/or magnetic resonance imaging of the head). Patients who had symptoms of transient ischemic attack were excluded. Based on neuroimaging, the strokes were classified as ischemic or hemorrhagic strokes. Ischemic strokes were further subdivided based on large artery strokes and small artery strokes irrespective of their etiologies. Large artery strokes were further subdivided depending on their anatomical vascular distribution such as anterior cerebral artery (ACA), middle cerebral artery (MCA), and posterior cerebral artery (PCA) stroke. Hemorrhagic strokes were assessed for their locations. The study was approved by the institutional ethics committee. Informed written consent was obtained from all individuals before inclusion in the study.

All study participants underwent analysis in terms of the following:

Demographic assessment

a. Patient's age, gender, side affected, and type of stroke at the time of admission
b. Neurological status at the time of admission according to the National Institutes of Health Stroke Scale (NIHSS)[3] in ischemic stroke and intracerebral hemorrhage (ICH) score in hemorrhagic stroke.

Clinical assessment

a. Assessment of the Functional Independence Measure (FIM) Scale scores and modified Rankin Scale (mRS) scores at admission
b. Presence of risk factors/comorbidities such as hypertension (HTN), diabetes mellitus (DM), diabetes + HTN, alcohol, and smoking.

Assessment of serum levels of brain-derived neurotrophic factor

BDNF blood concentration on the 1st day of admission was estimated. Blood was collected in an amount of 2 mL and allowed to stand for 1 h at room temperature. The sample was then centrifuged, and serum was separated by centrifugation of blood sample at 1500 g and stored at – 80°C for further processing.[4] Serum concentration of BDNF was assessed by enzyme-linked immunosorbent assay (ELISA) using a double-sandwich human BDNF ELISA kit (Raybiomed, Boster). Seven standard concentrations (2000, 1000, 500, 125, 62.5, 31.2, and 0 ng/ml) were assessed for corresponding optical density (OD) values, and a standard curve was generated. OD values of samples were read by the ELISA reader at wavelength 450 nm.

The mean concentration of BDNF in the whole group was assessed as well as in subgroups formed according to age (<55 and >55 years), gender, neurological status on the 1st day of stroke by NIHSS (<6 points vs. >6 points),[3] functional status by mRS (0–2 and 3–5),[3] and FIM (<60 and >60). We also assessed serum levels of BDNF in healthy controls ($n = 40$) to compare the BDNF levels with stroke patients. Healthy controls were selected from the staff in the department of neurology in our institute and caregivers of admitted patients in the same department. Proper history for any risk factor for stroke was taken for precise results. The age of healthy individuals was between 20 and 65 years.

Statistical analysis

All statistical analyses were performed using IBM SPSS 20.0, Armonk, New York, United states. An independent *t*-test was applied to compare the scores between groups. Multivariate analysis (linear regression) was performed to identify the independent factor responsible for poststroke disability. One-way ANOVA was applied to compare the mean scores in more than two groups. GraphPad PRISM was used to plot the figures. $P < 0.05$ was considered as statistically significant.

Results

The study involved 208 patients with confirmed stroke. The detailed demography is mentioned in Table 1. Most of the patients were in the seventh decade. The mean age of the patients was 55.29 years ± 11.06 (range from 18 to 75 years). The male-to-female ratio was 1.5:1. HTN was the most common risk factor (80%), followed by DM (50%), and 30% had both diabetes and HTN. Other risk factors included were dyslipidemia (29%), alcohol consumption (23%), and smoking (42%). The mean NIHSS score was 10.54 (1–25). Among patients with ischemic stroke, 91.4% had MCA stroke, 5.7% had ACA stroke, and 2.8% had PCA stroke. Among

Table 1: Demographic details of participants

Variables	Participants with stroke (*n*=208)
Age (years) (SD)	55.29 (11.06)
Sex (female/male)	82/126
Side affected (left/right)	129/79
Type of stroke	
Ischemic stroke	84
Large artery stroke (%)	70
MCA stroke	64 (91.4)
ACA stroke	4 (5.7)
PCA stroke	2 (2.8)
Lacunar stroke (%)	14 (16.6)
Hemorrhagic stroke (%)	124
Putamen	54 (43.5)
Thalamus	40 (32.2)
Pontine	22 (17.7)
Lobar	8 (6.4)
NIHSS (1-42) (*n*=84) (40%)	
Mild (1-4)	17
Moderate (5-14)	60
Severe (15-25)	7
ICH scoring (*n*=124) (60%)	
1	66
2	44
>3	14
Recurrent stroke	14
Expired	4
Risk factors, *n* (%)	
HTN	166 (80)
DM	104 (50)
Both HTN+DM	62 (30)
Alcoholic	48 (23)
Dyslipidemia	60 (29)
Smoking	87 (42)
Functional status	
mRS (0-6), *n*	
1	10
2	11
3	24
4	158
5	5

NIHSS: National Institute of Health Stroke Scale, HTN: Hypertension, DM: Diabetes mellitus, ICH: Intracerebral hemorrhage, MCA: Middle cerebral artery, ACA: Anterior cerebral artery, PCA: Posterior cerebral artery, SD: Standard deviation, *n*: Number of cases

patients with ICH (n = 124), 54 had putaminal bleed, 40 had thalamic pathology, 22 had pontine bleed, and 8 had lobar bleed. Among patients with ischemic stroke, 70 had large vessel infarct and 14 had small vessel (lacunar) infarct.

The mean BDNF level in patients with stroke at the time of admission was 9.93 ± 4.04 ng/mL (range, 0.21–19.47 ng/ml). The control value of BDNF levels in age- and sex-matched healthy individuals was 19.80 ± 4.0 ng/ml. Thus, a significant drop in BDNF level was observed in patients with stroke ($P < 0.05$). A significant difference in the levels of BDNF was observed in comparing the stroke patients and healthy individuals of age less than and more than 55 years and NIHSS stroke <6 and >6. BDNF levels in patients (with different risk factors) with diabetes, HTN, DM + HTN, alcohol, and smoking history were 8.8 ± 4.04 ng/ml, 8.86 ± 4.68 ng/ml, 8.65 ± 3.26 ng/m, 8.51 ± 4.26 ng/ml, and 8.9 ± 3.4 ng/ml, respectively [Table 2]. A decline in BDNF levels was observed in accordance with the severity of stroke in both ischemic and hemorrhagic stroke, with the least level being in severe stroke (NIHSS >15 and ICH >3) [Figure 1]. The mean BDNF level in patients with ischemic stroke was 10.73 ± 4.02 ng/ml and hemorrhagic stroke was 9.29 ± 4.04 ng/ml. Furthermore, the patients having recurrent stroke and those who expired showed a maximum decline in BDNF levels [Figure 1]. BDNF levels in patients with duration of stroke (from the time of ictus) are shown in Figure 2. It shows a linear decline in the BDNF levels with duration of stroke at the time of measurement, but the difference did not show statistical significance. We observed a significant difference in the BDNF levels in patients with FIM scores <60 and >60. A similar finding was there in mRS scores and BDNF levels. Patients with mRS score 0–2 showed significantly higher levels of BDNF than those who have mRS score range 3–5 [Figure 3]. We also observed that DM (P = 0.001), both diabetes + HTN (P = 0.001), alcohol (P = 0.003), and smoking (P = 0.002) were significant risk factors for poor outcome after stroke, whereas alone HTN (P = 0.8) was not a significant risk factor to affect stroke outcome.

Discussion

Globally more than 15 million people worldwide experience strokes each year, which equals to stroke every 2 s. Recent advances in the hyperacute management of ischemic stroke have led to a decline in stroke mortality, but the morbidity continues to increase. The epidemiological shift of the stroke disease will have a continuous rise in persons with disability which often goes unrecognized and has a high overall economic and social burden. Improvement in recovery and long-term outcome should be an urgent scientific goal and/or efforts should be made to recognize markers for stroke improvement. The basic elements of neural repair in animal models reflect dendritic branching, axonal sprouting, neurogenesis, synaptogenesis, and gliogenesis. Axonal sprouting in the brain is associated with improved poststroke outcome. The definitive evidences regarding these restorative processes in humans are scarce. However, markers which suggest neurogenesis, gliogenesis, and axonal sprouting after stroke or brain injury have been recognized in humans in perilesional brain tissue. Poststroke regenerative process may be a consequence of signaling of excitatory neurotransmitter like glutamate

Table 2: Levels of brain-derived neurotrophic factor in different groups

Variables	BDNF levels		*P*
BDNF (ng/ml)	In patients with stroke (*n*=208)	In healthy controls (*n*=40)	<0.01**
	9.93±4.04	19.80±4.0	
Age	<55 (*n*=90)	<55 (*n*=32)	<0.01**
	10.41±3.21>55 (*n*=118) 9.81±4.48	22.97±3.8>55 (*n*=8) 15.42±4.9	<0.01**
NIHSS	<6 (*n*=28)	>6 (*n*=180)	<0.01**
	13.8±4.1	9.8±3.8	
Risk factors			
Diabetes	Yes (*n*=104)	No (*n*=104)	0.001**
	8.8±4.0	11.08±3.9	
Hypertension	Yes (*n*=166)	No (*n*=42)	0.06 (NS)
	8.86±4.68	10.39±3.7	
DM+HTN	Yes (*n*=62)	No (*n*=146)	0.002 **
	8.65±3.26	10.64±4.08	
Alcoholics	Yes (*n*=48)	No (*n*=160)	0.003**
	8.51±4.26	8.80±0.669	
Smoking	Yes (*n*=87)	No (*n*=121)	0.001**
	8.9±3.4	10.9±3.4	

P: Significance, **<0.01, NS: Not significant, BDNF: **B**rain-**D**erived **N**eurotrophic **F**actor, NIHSS: National Institute of Health Stroke Scale, DM: Diabetes mellitus, HTN: Hypertension, *n*: Number of cases

and its counteraction by inhibitory neurotransmitter like GABA. These altered neuronal activities may result in change in activity of growth factor (GF) such as BDNF through epigenetic mechanism. Thus, the level of BDNF may indirectly reflect the potential for improvement in stroke outcome. With this aim, we tried to measure BDNF levels in patients with acute stroke and tried to correlate them with different types of stroke, demographic factors, and risk factors.

In our study, BDNF was significantly lower in patients following acute stroke [Table 2]. A similar finding was observed by Stanne *et al*.[5] A fall in BDNF is probably due to the downstream induction of BDNF secondary to altered neuronal excitability with downstream signal in excitatory neurotransmitters.[6] We observed that BDNF levels fall in accordance with severity of stroke. Whereas the BDNF level was 16.06 ± 2.02 ng/ml in mild ischemic stroke, it was 9.26 ± 2.18 ng/ml in severe stroke [Figure 1]. The correlation was even more marked in patients with hemorrhagic stroke, where patients with ICH score 1 have BDNF 14.1 ± 3.7 ng/ml, while those with ICH score 3 and above had mean BDNF levels 5.3 ± 2.3 ng/ml. Qiao *et al*.[7] have stated that larger infarct volumes were associated with lower levels of BDNF at admission. They found a negative correlation between serum BDNF level and diffusion-weighted imaging infarct volume ($r = -0.363$; $P = < 0.001$).[7] We observed no correlation with level of BDNF and duration of stroke in acute stage ($P > 0.05$) [Figure 2]. One possible cause may that neurotrophin expression and secretion is an

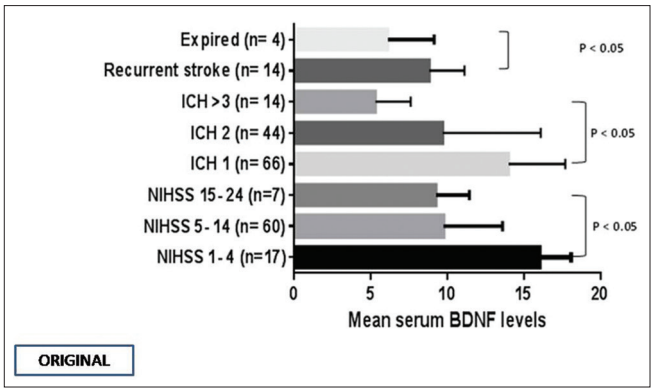

Figure 1: Levels of brain-derived neurotrophic factor in healthy controls, ischemic stroke, and hemorrhagic strokes of different intensity

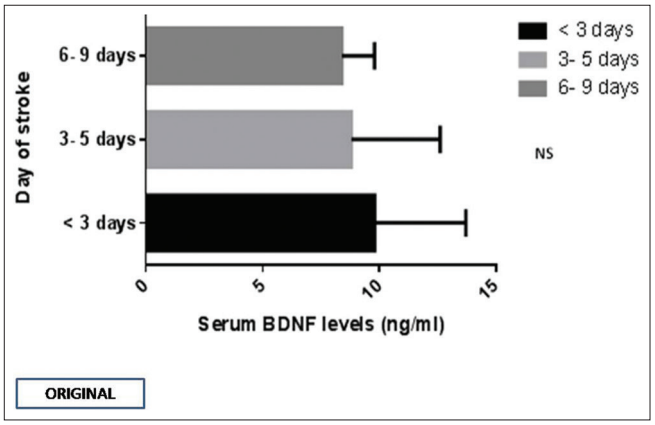

Figure 2: Levels of brain-derived neurotrophic factor according to the duration of stroke at the time of admission

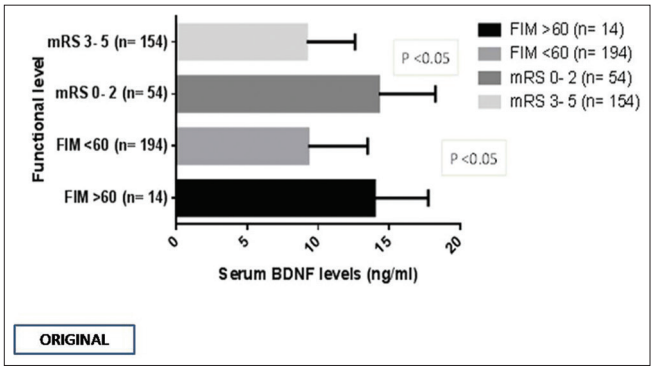

Figure 3: Levels of brain-derived neurotrophic factor in stroke patients who showed better improvement (functional independence measure >60, modified Rankin Scale: 0–2) and those with poor improvement (functional independence measure <60; modified Rankin Scale: 3–5)

activity-dependent mechanism.[8] Similar results were found by Rodier *et al*.[9] in an animal study. They assessed BDNF levels in patients with stroke at admission and after days 1, 7, and 90. No significant difference was found in the levels in groups.

Among the risk factors, fall in BDNF levels is mostly seen in diabetics, alcoholics, and smokers. No fall was

observed in hypertensives though a significant fall in BDNF level was observed in patients having both diabetes and HTN. Low BDNF concentrations have also been observed in patients with metabolic syndrome.[10] Diabetes is one of the vital comorbidity risk factors that have been reported to be associated with occurrence, poor outcome, and recurrence in stroke patients.[11] However, the underlying mechanism is not fully understood. Secretion of neurotrophic factors such as BDNF by the cerebral endothelium is suppressed in diabetes. Consequently, neuroprotective deficits make neurons more vulnerable to injury.[12] Age-adjusted incidence rates suggest that diabetic patients are three times more likely to have a stroke compared to nondiabetic patients.[13] Diminished cognitive abilities are found in patients with type 1 diabetes, whereas type 2 diabetes is known to affect learning and memory as well.[14]

DM and HTN both collectively are major risk factors for stroke. Some researchers claim that treatment with BDNF lowers blood glucose levels in diabetic mice models.[15] Similarly, Yamanaka *et al*.[16] demonstrated that treatment with BDNF helps in preventing an age-related increase in blood glucose and development of diabetes in prediabetic mice. The data derived from animal experiments suggest that exogenous BDNF administration shows its antidiabetic and antilipidemic effects. Serum BDNF levels were also significantly improved in patients without diabetes and HTN. Smoking is also a risk factor for stroke. However, studies are in favor of raised BDNF levels in smokers.[17] However, these studies were carried out on the participants without any history of stroke. In our study, we observed more decline in BDNF levels in smokers as compared to nonsmokers. Durazzo *et al*.[18] have stated that chronic smoking is associated with inferior performance on the measures of general intelligence, visuospatial learning and memory, and fine motor dexterity. One possible reason of poor quality of life in stroke patients with smoking history is that smoking leads to cognitive decline.[19] Negative influences of smoking have been observed on muscle and tendons. The loss of bone mineral content has increased the risk and incidence of fractures and is the best known negative consequence. Nicotine has direct toxic effects on osteoclast and osteoblast activity. The indirect effects of nicotine are indirect actions on sex hormone and adrenocortical hormones, Vitamin D, intestinal calcium absorption, vessels, and oxygen supply.[20] These changes in the musculoskeletal system further may interrupt the formation and functions of BDNF indirectly.

Alcohol intake suppresses BDNF expression and results in the decrease of pERK1/2 and Bcl-2 in the hippocampus, which are its downstream molecules. Alcohol intake may lead to a reduction in hippocampal cell proliferation through inhibition of the BDNF-ERK signaling pathway.[21] On comparing levels of BDNF in alcoholic and nonalcoholic stroke patients, we found lower BDNF levels in alcoholic stroke patients as compared to nonalcoholic stroke patients. Alcoholic stroke patients did not show significant even at 2 weeks of intervention. Our results are in favor of findings by Logrip *et al*.[22] that GFs, long studied for their involvement in neuronal development and plasticity, also regulate responses to drugs of abuse, including alcohol.

A significant decline in the levels of BDNF after stroke has been observed. The decline has been noted in accordance with the severity of stroke. The levels of BDNF are also affected by low NIHSS (<6) at the time of admission. Risk factors (DM, alcohol consumption, both diabetes and HTN, and smoking) associated with stroke negatively affect the levels. However, a significant difference in levels was not found in hypertensives and nonhypertensives at the time of admission.

Financial support and sponsorship

This study was financially supported by the Department of Science and Technology, New Delhi, India, Grant no. SR/WOS-A/LS-410/2013.

Conflicts of interest

There are no conflicts of interest.

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