Post-stroke BDNF concentration changes following proprioceptive neuromuscular facilitation (PNF) exercises

Poonam Chaturvedi[1], Ajai Kumar Singh[1], Vandana Tiwari[2], Anup Kumar Thacker[1]

Departments of [1]Neurology and [2]Biochemistry, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, UP, India

Abstract

Background: Brain-derived neurotrophic factor (BDNF) plays an important role in repairing normal as well as in the injured brain. Physical exercise may have a positive impact on the release of BDNF. **Objective:** PNF is a neurophysiological approach that facilitates the stimulation of central and peripheral nervous systems. In this study, our aim was to assess the levels of BDNF as well as functional recovery before and after the intervention of PNF in patients with acute stroke. **Methods:** A total of 208 patients with first time confirmed stroke were recruited and assessed for stroke severity, type, mini-mental state exam (MMSE), functional independence measure scale, and BDNF levels before and after PNF intervention. BDNF levels were also assessed in healthy individuals for control values. **Results:** A significant decline in levels of BDNF was observed after in stroke. BDNF levels in patients (with different risk factors) with diabetes, hypertension and DM+ HTN, alcohol, and smoking history were 8.8 ± 4.04 ng/mL, 8.86 ± 4.68 ng/mL, 8.65 ± 3.26 ng/mL, 8.51 ± 4.26 ng/mL, and 8.9 ± 3.4 ng/mL, respectively. 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). Despite the type of stroke and the presence of risk factors, a significant improvement in BDNF levels and FIM scale scores was seen in all subjects who received PNF exercises. **Conclusion:** Thus, PNF is efficient in improving functional level in acute stroke irrespective of the type of stroke and risk factors.

Keywords: Brain-derived neurotrophic factor, proprioceptive neuromuscular facilitation, risk factors, stroke

Introduction

Consequences of stroke are grave with one-third of the affected population are left with a decline in functional ability. Neurorehabilitation remains the only hope in restoring the functional capacity of the individual with continuous efforts on preventing the risk factors following the hyperacute period of the stroke. An effort to take advantage of the critical period where methodology, timing, and intensity will procure maximum neuro- rehabilitation to augment the biological mechanism of post-stroke plasticity, can result in a better outcome. PNF is one such rehabilitation technique where multiple sensory stimulation techniques combine to improve the functional outcome of patients with stroke. Proprioceptive neuromuscular facilitation (PNF) is a concept of treatment for motor learning and motor control[1] and it works by stimulation of muscle and joint proprioceptors[2] using the principles such as manual contact, body position, stretch, manual resistance, irradiation, joint facilitation, timing of movement, pattern of movement visual cues, and verbal input. Among the PNF's principles, irradiation[3] principle is based on the fact that stimulation of strong and preserved muscle groups produces strong activation

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of injured and weak muscles, facilitating muscle contraction.[4] With this aim in mind, we subjected our patients of acute stroke to PNF and its effect on the various outcome measures was studied following stroke. We also estimated that brain-derived neurotrophic factor (BDNF), which has an important role in brain plasticity and is a key molecule for memory in healthy as well as following focal CNS damage,[5] was correlated with clinical demographic and various functional outcome.

Subjects and Methods

The prospective cohort study (in the period from November 2014 to April 2018) involved 208 patients of a stroke aged 18 to 75 years with acute stroke. The diagnosis of stroke was based on neuroimaging procedures (CT and/or MRI of the head). Based on neuroimaging, the strokes were classified as ischemic or hemorrhagic strokes. Ischemic strokes were further subdivided as large artery strokes and small artery strokes irrespective of their etiologies. Large artery strokes were further subdivided depending upon 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.

Subjects were excluded according to the following criteria:

Transient ischemic attack (TIA), recurrent stroke, aphasia, very severe stroke, cognitive impairment (MMSE < 16), fracture, amputation, pregnancy, multiple organ failure, patients with functional impairment before the stroke, patients with psychiatric illness such as bipolar disorder and movement disorder such as Parkinsonism. The study was approved by the institutional ethics committee (September, 2015). Written informed consent was obtained from all patients or their legal relatives before inclusion into the study.

All study subjects underwent analysis in terms of the following:
1. *Demographic assessment*
   a. Patient's age, gender, the side affected, and type of stroke at the time of admission.
2. *Neurological status at the time of admission according to NIHSS (in ischemic stroke)[6,43] and ICH in (hemorrhagic stroke)*
3. *Clinical assessment*
   a. Assessment of FIM, mRS, and BI at admission (before PNF) and after 6 months.
   b. Presence of risk factors/comorbidities such as 1) hypertension, 2) diabetes mellitus, 3) diabetes + hypertension, 4) alcohol, and 5) smoking.

3. *Assessment of serum levels of BDNF*

BDNF blood concentration on the first day of admission (before PNF) was estimated. Blood was collected in an amount of 2 mL from the antecubital vein and allowed to stand for 1 hour at room temperature. The sample was then centrifuged at

1500 g and serum was separated and stored at -80°C for further processing.[7] The serum concentration of BDNF was assessed by ELISA (Enzyme-Linked Immunosorbent Assay) using a double sandwich human BDNF ELISA kit (Raybiomed Pvt. Ltd., Boster). Seven standard concentrations (2000, 1000, 500, 125, 62.5, 31.2, and 0 ng/mL) were assessed for corresponding OD (optical density) values and a standard curve was generated. OD values of samples were read by the ELISA reader at wavelength 450 nm.

Mean concentration of BDNF in the whole group was assessed as well as in subgroups formed according to age (<55 years and > 55 years), gender, type of stroke, and their further subtypes, risk factors such as T2DM, hypertension, both DM + HTN, alcoholics and smokers.

Procedure for PNF intervention

The intervention of PNF was given to all patients; from the day of hospitalization following a set protocol of PNF (30 min twice daily, 5 days a week for 2 weeks) and the patients were assessed after 6 months. PNF intervention was started in a proximal-to-distal direction.

- *PNF for neck:*
Patient's position: Supine lying
   - Therapist position: on the head side of the patient
   - Hand placement: one hand holding chin and another hand on the occiput.
   - Command: D1 flexion: "pull your chin in" and "look at your left hip."
     D1 extension: "lift your chin" and then "lift your head to look above."

The same procedure is repeated for flexion and rotation to the right; extension and rotation to the left D2 flexion and extension.

- *PNF for scapula:*
Patient's position: Side-lying with the affected side up.
Therapist position: standing behind the patient.
   - *Anterior elevation :* Command: "Shrug your shoulder up toward your nose.
   - *Posterior depression:* Command: Command. "Push your shoulder blade down to me".
   - *Posterior elevation:* Command: "Pull your shoulder blade down toward your navel."
   - *Anterior depression :* *Command:* "Shrug your shoulder up."

- PNF for pelvis
   Patient's position: Same as the scapula
   Therapist position: standing behind the patient
   Grip: The fingers of one hand grip around the crest of the ilium.
   - *Anterior elevation :* Command: Shrug your pelvis up.
   - *Posterior depression:* Command: Sit into my hand.
   - *Posterior elevation:* Command: Push your pelvis up and back

- *Anterior depression :* Command: Push your knee into my hand

- PNF for trunk:
  a) *Alternating isometrics*
    - Patient's position: Sitting on the edge of the plinth with feet resting on the floor
    - Therapist position: Stand either behind or in front of the patient with hands resting on both shoulders of the patient.
    - Command: "Do not let me push you back" for trunk flexors.
      "Do not let me pull you forward" for trunk extensors.
      "Do not let me push you sideways" for lateral flexion of the trunk.
  b) *Rhythmic stabilization:* Patient and therapist position as above
    - Hand placement: One hand on shoulder anteriorly and another hand on another shoulder posteriorly to rotate the trunk.
    - Command: Do not let me rotate your trunk.

- Extremity patterns were started once tone starts developing in extremities (even flicker contraction of muscles around shoulder joint).
  Pattern: D1 and D2 flexion and extension (*refer to* Table 1).

Results

The study involved 208 patients with confirmed stroke. The detailed demography has been mentioned in Table 2. Most of the patients were in the seventh decade. The mean age of the patients was 55.29 years ± 11.06 (range from 18- 75 years). Male to female ratio was 1.5:1. Hypertension was the commonest risk factor (80%) followed by diabetes mellitus (50%) and 30% had both diabetes and hypertension. Other risk factors included were dyslipidemia (29%), alcohol consumption (23%), and smoking (42%).

We observed a significant difference in the FIM scores in patients with an increase in the severity of stroke [Table 3, Figure 1]. There was significant improvement in FIM scores in all subjects ($P < 0.05$) after PNF intervention but patients with mild

stroke (NIHSS 1-4) (FIM = 120 ± 5.97) and ICH score 1 (FIM = 107 ± 21.53) were almost independent after 6 months. However, the improvement in hemorrhagic stroke was better seen than those with ischemic stroke. The modified Rankin Scale Scores were also improved in all cases but, while observing the severe stroke, better improvement in hemorrhagic stroke than in ischemic stroke was observed. The improvement in the FIM score was less in patients with moderate (NIHSS 5–15) stroke, severe stroke (NIHSS >15) and ICH score 2 and >3 as compared to mild stroke. On analysis of Barthel's Index in all subjects, we found no significant difference in the scores ($P > 0.05$). The improvement was equal in mild and moderate stroke. However, patients with ischemic stroke gained better scores than patients with hemorrhagic stroke [Table 3].

On the estimation of BDNF, a significant increase in the levels in both ischemic, as well as hemorrhagic stroke, was observed following PNF and this increase was observed irrespective of the severity of stroke. It was also noted that the increment in the BDNF level was more marked in those patients who have a severe hemorrhagic stroke than those with severe ischemic stroke. 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 2, Table 4]. The increment in BDNF levels following PNF was observed in all patients irrespective of the day of the stroke. However, the difference was maximally observed in those where PNF was given after 5 days [Table 5].
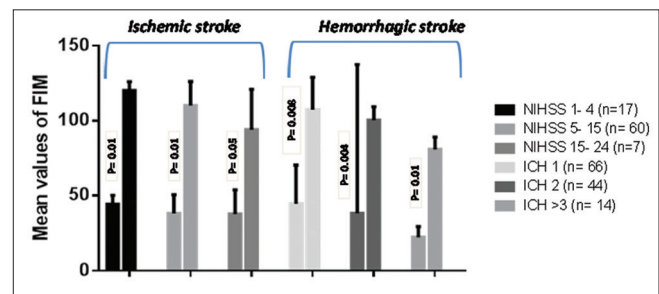

Figure 1: FIM scores in patients based on the type and severity of stroke at admission and after PNF (6 months)

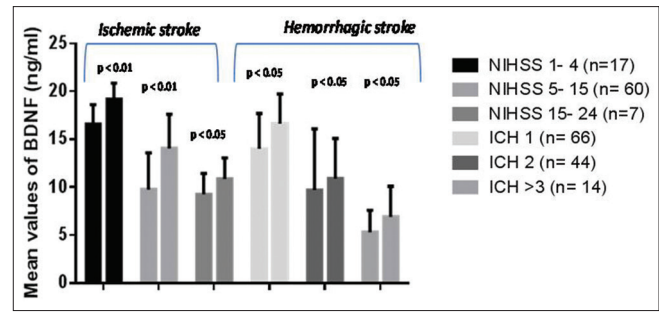| Table 1: Patterns and techniques followed for PNF intervention in acute stroke		
Parts of body	**Techniques (T) and patterns (P) used**	**Effects**
Neck	Flexion with rotation to the right (P)	Increase neck stability
	Extension with rotation to the left (P)	Improved trunk stability
	Flexion with rotation to the right (P)	
	Extension with rotation to the left (P)	
Trunk	Alternating isometrics (T)	Increases trunk stability
	Rhythmic stabilization (T)	The improved tone in Shoulder musculature
Scapula and pelvis	Rhythmic initiation (T)	Strengthening of shoulder muscles
	Slow reversals (T)	The improved tone in muscles of extremities
Upper extremity	Rhythmic initiation (T)	Improved strength in muscles
and lower extremity	Flexion-adduction-external-rotation (D1 flexion) (P)	Improved coordination
	Extension- abduction-internal rotation (D1 extension) (P)	Improvement in functional activities
	Flexion- abduction- external rotation (D2 flexion) (P)	Improvement in gait
	Extension- adduction- internal rotation (D2 extension) (P)	

Table 2: Demographic details of subjects

Variables	Subjects 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*) (%)	
Hypertension (HTN)	166 (80%)
Diabetes mellitus (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=Intra Cerebral Hemorrhage, MCA=Middle Cerebral Artery, ACA=Anterior Cerebral Artery, PCA=Posterior Cerebral Artery, SD=Standard Deviation, *n*=Number of cases

Figure 2: BDNF levels in patients based on the type and severity of stroke before and after PNF

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). A significant difference in the levels of BDNF was observed on comparing the stroke patients and healthy individuals of age <55 years and >55 years, females and males ($P = 0.005$) but irrespective of side affected. Patients with ischemic stroke of the lacunar type exhibited more BDNF levels before and after PNF than patients with large artery ischemic stroke. Patients having pontine bleed had better BDNF levels 13.01 $\pm$ 3.83 ng/mL) with the least levels in lobar bleed (6.82 $\pm$ 2.67 ng/mL). However, improvement in BDNF levels was seen in all cases. BDNF levels in patients (with different risk factors) with diabetes, hypertension, and DM + HTN, alcohol and smoking history were 8.8 ± 4.04 ng/mL, 8.86 ± 4.68 ng/mL, 8.65 ± 3.26 ng/mL, 8.51 ± 4.26 ng/mL, and 8.9 ± 3.4 ng/mL, respectively [Table 6]. A significant improvement was seen in all cases with risk factors

except in alcoholics [Table 6]. No significant difference in BDNF level was found in patients with and without hypertension. The risk factors that significantly affected the stroke outcome were diabetes, both hypertension and diabetes, alcohol consumption and smoking [Table 7].

Discussion

We included 208 patients of acute stroke and they were subjected to PNF from the day of hospitalization following a set protocol of PNF (30 min twice daily, 5 days a week for 2 weeks) and the patients were assessed at 6 months. Simultaneously BDNF levels were also measured before initiation of PNF and at 6 months to note the changes in BDNF levels. BDNF is lowered in patients of acute stroke.[8] The fall in BDNF is probably due to the downstream induction of BDNF secondary to altered neuronal excitability with the downstream signal in excitatory neurotransmitters.[9,10] We observed that BDNF levels fall in accordance with the 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 2]. The correlation was even more marked in patients with hemorrhagic stroke where those 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. According to Qiao *et al.* (2017) larger infarct volumes are associated with lower levels of BDNF at admission (r = -.363; $P = <.001$).[11] We observed no difference with the level of BDNF and the duration of a stroke at least in the acute stage [Table 5]. Similar results were found by Rodier *et al.* (2015) in an animal study in which no significant difference found in the BDNF levels in subjects with stroke at admission and after day 1, 7, and 90.[12]

All those patients who received PNF, improvement in FIM as well as in mRS and Barthel's Index were significantly improved. The improvement is more marked in those patients with mild stroke irrespective of it being an ischemic and hemorrhagic stroke. The degree of improvement in these parameters was more marked in patients having a hemorrhagic stroke compared to those with ischemic stroke. Though it did not reach a statistically significant level. It is well recognized that the tissue damage is greater in patients with an ischemic stroke rather than those with hemorrhagic. On the estimation of BDNF in these patient's, a similar observation was made where the maximum elevation of

Table 3: Functional levels before and after PNF in ischemic and hemorrhagic stroke

Variables	FIM in ischemic stroke			FIM in hemorrhagic stroke		
	FIM before PNF	FIM after PNF	ICH score	FIM before PNF	FIM after PNF	
Mild (1-4) (*n*=17)	44.39±5.89	120±5.97	1 (*n*=66)	44.72±25.75	107.35±21.53	
Moderate (5-14) (*n*=60)	38.18±12.52	110±16.0	2 (*n*=44)	38.28±9.81	100.25±9.0	
Severe (15-24) (*n*=7)	37.75±16.23	94±26.8	>3 (*n*=14)	22.85±7.15	90.85±8.2	
P	0.092	0.001**		0.021**	0.030*	
	mRS in ischemic stroke			mRS in hemorrhagic stroke		
	mRS before PNF	mRS after PNF	ICH score	mRS before PNF	mRS after PNF	
Mild (1-4) (*n*=17)	3.37±0.95	0.80±0.57	1	3.89±1.21	1.10±0.93	
Moderate (5-14) (60)	3.52±0.96	1.24±0.85	2	3.54±0.50	1.68±0.62	
Severe (15-24) (7)	3.97±0.52	2.0±0.93	>3	4.27±0.79	1.95±0.21	
P	0.060	0.001**		0.012**	0.001**	
	BI in ischemic stroke			BI in hemorrhagic stroke		
	BI before PNF	BI after PNF	ICH score	BI before PNF	BI after PNF	
Mild (1-4) (*n*=17)	45.0±30.0	97.86±3.9	1	41.0±25.0	96.0±5.47	
Moderate (5-14) (*n*=60)	27.14±17.0	90.50±10.5	2	18.57±15.11	89.64±15.62	
Severe (15-24) (*n*=7)	26.67±11.5	72.5±16.9	>3	12.50±11.58	86.87±10.66	
P	0.002**	0.04*		0.023**	0.47	

FIM: Functional Independence measure, mRS: modified Rankin Scale, PNF: Proprioceptive Neuromuscular Facilitation, BI: Barthel's- Index, *P < .05 ** P < .01

Table 4: BDNF levels in ischemic and hemorrhagic stroke

BDNF in ischemic stroke				BDNF in hemorrhagic stroke			
(Range of NIHSS) (*n*)	BDNF before PNF	BDNF after PNF	P	ICH score	BDNF before PNF	BDNF after PNF	P
Mild (1-4) (17)	16.06±2.02	19.19±1.67	<0.01**	1	14.0±3.7	16.64±3.1	<0.05*
Moderate (5-14) (60)	9.75±3.85	14.03±3.55	<0.01**	2	9.7±6.4	10.9±4.2	<0.05*
Severe (15-24) (7)	9.26±2.18	10.87±0.57	<0.05*	>3	5.3±2.3	6.9±1.2	<0.05*

*= significance level <.05, **= significance level <.01, SD=Standard deviation, S.BDNF=Serum brain derived neurotrophic factor, NIHSS=National Institute of Health Stroke Scale

Table 5: Levels of BDNF according to the duration of stroke

	BDNF in ischemic stroke		BDNF in hemorrhagic stroke	
Day of stroke	Before PNF	After PNF	Before PNF	After PNF
<3 days (*n*=54)	9.2±2.9	13.3±3.3	9.8±4.9	14.5±4.2
3-5 days (*n*=35)	7.4±1.0	10.8±3.1	10.5±4.0	11.5±3.9
>5 days (*n*=119)	8.96±2.0	16.65±2.6	8.7±1.9	15.4±0.60
P	0.433	0.017**	0.66	0.78

BDNF: Brain-derived neurotrophic factor, PNF: Proprioceptive neuromuscular facilitation, **P < .01

BDNF was noted in mild ischemic stroke compared to those with hemorrhagic stroke. It has been observed that following acute stroke, alteration of neuronal brain activity can be reversed by a homeostatic increase in neuronal excitability. Enhanced glutamate signaling through AMPA receptors secondary to downstream induction of BDNF has been shown to alter neuronal excitability.[9,13]

Nonpharmacological approaches have been shown to enhance structural plasticity by altering cortical excitability and inhibitory balance. In a mouse model, direct current stimulation to brain augmented synaptic plasticity through BDNF dependent mechanism.[14-16] Though data in human studies poorly understood elevation of BDNF post-PNF can reflect this mechanism. We observed elevation of BDNF following PNF irrespective of the day of administration of PNF in these patients. Though a maximum elevation in BDNF was noted in those where it was started after day 5 of stroke onset. This means that PNF is effective in all stroke irrespective of the day of PNF.

We observed difference in level of BDNF in patients >50 years of age (*P* =0.005) and in females (*P* =0.005). According to Bathina *et al.* (2014), BDNF levels are decreased with increasing age and are found more in females as compared to males of the same age.[17] Whereas no significant difference was there in different hemispheric stroke (*P* =.08). Patients with lacunar stroke showed a significantly higher level of BDNF both before and after PNF intervention than patients with large artery stroke (*P* =.001). Amongst the patients with hemorrhagic stroke, the higher levels of BDNF were achieved by the subjects with putaminal bleed (9.77 ± 4.28 ng/mL to 17.89 ± 2.42, *P* =.001) whereas the lowest rise in levels were seen in lobar bleed (6.82 ± 2.67 to 9.34 ± 1.42, *P* =.001).

Among the risk factors, the fall in BDNF levels is mostly seen in diabetes, alcoholics, and smokers. No fall was observed in hypertensive patients. Though a significant fall in BDNF levels was observed in patients having both diabetes and hypertension [Table 6]. Low BDNF concentrations have also been observed in patients with metabolic syndrome,[18] atrial fibrillation[19], and acute coronary syndromes.[20] Diabetes is one of the vital comorbidity risk factors reported to be associated with the occurrence, poor outcome, and recurrence in stroke patients.[21-23] Secretion of neurotrophic factors

Table 6: BDNF levels and FIM scores before and after PNF in different cohorts

Variables	BDNF levels		P	FIM scores		P
	Before PNF	After PNF		Before PNF	After PNF	
In all subjects (*n*=208)	9.93±4.04	13.65±3.69	0.001[a] **			
Age						
<55 years (*n*=90)	10.41±3.21	14.10±4.68	0.005[b] **	38.9±16.9	107.1±15.9	0.218[b]
>55 years (*n*=118)	9.81±4.48	10.28±2.14		35.3±12.7	109.7±17.4	
Gender						
Males (*n*=126)	9.01±4.49	12.73±5.01	0.005[b] **	41.7±18.5	104.5±17.2	0.002[b]**
Females (*n*=82)	11.43±2.70	15.29±3.37		35.5±10.5	111.6±13.9	
Side affected						
Right (*n*=129)	10.29±4.11	12.84±4.22	0.08[b]	38.3±13.4	107.4±17.4	0.93[b]
Left (*n*=79)	9.99±3.90	14.60±4.48		38.1±17.8	107.5±15.5	
Ischemic stroke						
Large artery stroke (*n*=70)	9.44±4.41	10.84±3.31	0.001[b] **	40.5±12.2	105.5±22.4	0.007[b]**
Lacunar stroke (*n*=14)	12.12±3.83	16.26±3.31		38.9±13.1	115.4±8.5	
Hemorrhagic stroke						
Putaminal bleed (*n*=54)	9.77±4.28	17.89±2.42	0.001[b] **	38.8±21.8	105.9±17.4	0.005[b] **
Thalamic bleed (*n*=40)	10.60±2.65	10.58±3.12		32.5±10.7	107.6±10.2	
Pontine bleed (*n*=22)	13.01±3.83	16.17±4.16		48.0±13.5	109.0±9.4	
Lobar bleed (*n*=8)	6.82±2.67	9.34±1.42		41.8±12.8	91.2±8.0	
Risk factors						
Hypertensives (*n*=166)	8.86±4.68	11.35±2.9		38.6±17.1	105.3±16.6	
Nonhypertensives (*n*=42)	10.39±3.7	14.42±4.52	0.06[b]	35.8±9.7	119.5±3.9	0.01[b] **
Diabetics (*n*=104)	8.8±4.0	10.90±3.9		36.6±10.8	101.9±18.1	
Nondiabetics (*n*=104)	11.08±3.9	15.4±3.4	0.001[b] **	39.4±19.5	112.6±12.8	0.001[b] **
With Both HTN + DM (*n*=62)	8.65±3.26	9.71±1.60		36.4±11.5	94.3±16.5	
Without HTN + DM (*n*=146)	10.64±4.08	15.13±4.26	0.001[b] **	39.0±17.9	113.5±12.0	0.001[b] **
Alcoholics (*n*=48)	8.51±4.26	8.80±0.66		37.0±11.2	90.5±16.5	
Nonalcoholics (*n*=160)	10.34±3.96	14.77±2.88	0.001[b] **	38.5±17.3	112.2±12.6	0.001[b] **
Smokers (*n*=87)	8.9±3.4	10.89±4.26		38.6±15.1	101.7±18.3	
Nonsmokers (*n*=121)	10.9±3.4	15.29±3.46	0.001[b] **	37.9±16.9	111.3±13.4	0.001[b] **

HTN=Hypertension, DM=Diabetes Mellitus, [a]P value within the group, FIM: Functional Independence Measure Scale Scores, [b]P value in between group, ** P < .01

Table 7: Risk factors affecting the recovery after stroke

Risk factors	P (for BDNF as an outcome)
Diabetes Mellitus	0.001**
Hypertension	0.78
Both Diabetes and hypertension	0.001**
Alcohol	0.002**
Smoking	0.002**
Age	0.061
Sex	0.136

**P<0.01

by the cerebral endothelium, such as BDNF, is suppressed in diabetes and make neurons more vulnerable to injury,[24] in-hospital mortality, and slower recovery compared with nondiabetic individuals.[25-28] In our study, we observed significant improvement in functional activities (FIM) and BDNF levels but more improvement was seen in nondiabetics after 6 months. Paker *et al.* (2016) have indicated better improvement in functional activities in nondiabetic patients as compared to diabetic stroke patients.[29]

Diabetes mellitus and hypertension both collectively are major risk factors for stroke. Some researchers claim that BDNF treatment reported to lower blood glucose in diabetic models.[30] Similarly, Yamanaka *et al.* (2008) demonstrated that treatment with

BDNF prevents an age-related increase in blood glucose and the development of diabetes in prediabetic mice[31]. BDNF levels were also significantly improved in patients without diabetes and hypertension. Smoking is also a risk factor for stroke. However, studies are in favor of raised BDNF levels in smokers[32] but these studies were carried out on the subjects without any history of stroke. In our study, we observed more decline in BDNF levels in smokers as compared to nonsmokers. Durazzo *et al.* (2012) have stated that chronic smoking is associated with inferior performance on the measures of general intelligence, visuospatial learning, and memory and fine motor dexterity.[33] Negative influences of smoking have been observed on bone, muscle, and tendons. In bones, loss of mineral content and increased incidence of fractures occurs. Nicotine directly affects osteoblasts/osteoclasts activity, and indirect actions on vitamin D, adrenocortical hormones, oxygen supply to the vessels, and intestinal calcium absorption.[34] These changes in the musculoskeletal system further may interrupt the formation and functions of BDNF indirectly.

Alcohol intake suppressed BDNF expression and resulted in the decrease of its downstream molecules, pERK1/2 and Bcl-2, in the hippocampus. Alcohol intake may lead to reduced hippocampal cell proliferation through inhibition of the BDNF-ERK signaling pathway.[35] On comparing levels of BDNF in alcoholics and nonalcoholics stroke patients we found

lower BDNF levels in alcoholic stroke patients as compared to nonalcoholic stroke patients.[35,36]

On comparing FIM scores in different cohorts [Table 6], we observed equal improvement in the FIM scores in patients ($P = 0.218$). This suggests that PNF improves the functional activity in all age groups. According to the earlier studies, elderly patients are at higher risk of poor functional outcome, mortality, and prolonged hospital stay. In our study, we observed that there was equal recovery irrespective all these factors in the elderly. We compared functional activities in the right and left hemiplegics and both groups exhibited equal improvement ($P = 0.93$). The study was done by Fink *et al.* (2008) on 1644 placebo-treated patients, found no difference in functional outcome between the two hemispheres, which is in agreement with our findings.[37] There are also gender differences in various factors of stroke such as risk factors, clinical manifestations, mortalities, and functional outcomes, they have received attention only recently. However, the existence of gender differences in other stroke factors, such as functional outcome and mortality remains controversial.[38,39] We found better improvement in females after PNF intervention ($P =.002$). One cause of this finding may be that females are less prone to risk factors as compared to males and females have more levels of BDNF as compared to males. Hypertension can also have an impact on functional recovery. According to Bager *et al.* (2018), managing higher BP after the patients' arrival to the ward were associated with improved functional outcome, and reduced mortality, respectively.[40] In our study, the functional improvement was equal in both hypertensive and nonhypertensive subjects. This means the functional outcome is not affected by the presence of hypertension if PNF is given. Diabetes is a risk factor for both stroke and poor functional outcome. Diabetic patients had other difficulties such as muscle atrophy, pain, neuropathies, which may contribute to poor functional recovery. In our study, there was an improvement in FIM scores in all subjects, but more improvement was seen in nondiabetics compared to those without diabetes ($P = 0.001$). According to Jia *et al.* (2011)[23], DM had a significantly higher incidence of death, dependency, recurrent stroke at 3 and 6 months after stroke onset and is an independent risk factor for death or dependency. He found a significant correlation ($P < 0.05$) for age, sex, smoking, and alcoholism with stroke severity in ischemic stroke patients with diabetes. We also found similar results. In our study, the improvement was seen in both with and without DM+ HTN, but patients without DM+ HTN were more improved ($P = 0.001$). Smoking is also one of the risk factors for both stroke and poor outcome after stroke as well. Despite the compelling evidence that nicotine has beneficial effects, nicotine can be toxic under some circumstances. The balance between nicotine neuroprotection and toxicity depends upon the dose.[41] In our finding patients without a history of smoking had better and significant ($P =.001$) functional recovery ($P = 0.001$). In our study, patients with a history of alcoholism had a poor functional outcome. This poor recovery in functional activities may be due to low levels of BDNF in these cases. The recovery was better in nonalcoholics ($P = 0.001$). The acute and chronic effects of alcohol on bone, muscle and peripheral nerves include osteoporosis, osteonecrosis and traumatic fractures. In muscle, heavy drinking may cause rhabdomyolysis while chronic alcohol abuse may produce proximal myopathy. In peripheral nerves, acute alcohol intoxication may lead to pressure neuropathy and chronic abuse may cause peripheral neuropathy.[42]

The study reveals that PNF improves BDNF levels hence improve neuroplasticity. It should be recommended in all hospitals, clinics, and rehabilitation centers. Early improvement in the functional activities will reduce hospital stay, reduce expenditure, and burden on caregivers. BDNF is also associated with cognition, so a rise in BDNF levels will reduce the chances of depression and give clinicians a better stroke outcome.

Conclusion

BDNF levels are decreased in acute stroke. These levels are further declined in the presence of risk factors. PNF exercise can promote changes in central BDNF concentrations and promote functional recovery in acute stroke.

Limitations of the study

PNF is a standardized exercise and is a noninvasive method of intervention but it needs the attention of the patient. PNF improves functional activity but it cannot be given to the aphasic stroke patients and patients with cognitive impairment. Because these patients can not follow complex commands given by the therapist.

Recommendations and implications for future research

The results of our study have shown a positive association of BDNF with functional recovery and PNF exercises are efficient to raise the BDNF levels after stroke even in the presence of risk factors. In the future, if PNF intervention and intravenous BDNF are given simultaneously, then the stroke recovery can be improved to a great extent.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Conflicts of interest

There are no conflicts of interest.

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