Role of Brain Biomarker in Predicting Clinical Outcome in Hypertensive Cerebrovascular Ischemic Stroke

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Abstract This study was undertaken to evaluate the role of serum neuron specific enolase (NSE) in prediction of disability and neurological worsening in hypertensive ischemic cerebrovascular stroke. 80 hypertensive ischemic stroke patients diagnosed by a neurologist as per WHO definition along with radiological findings suggestive of cerebrovascular stroke and differentiating from hemorrhagic stroke and 60 controls having essential hypertension coming to hospital because of regular checkup or headache but with no neurological disease were included in the study. Neurological disability was assessed by NIHSS at the time of admission (within 72 h from the onset of stroke) and on 7th day after admission and cases were categorized into mild, moderate and severe disability. Venous blood samples were drawn within 72 h from the onset of symptoms. The samples were processed as per the laboratory protocol. The serum NSE samples were analyzed using an enzyme immunoassay based on the sandwich technique. We observed raised serum NSE in hypertensive ischemic stroke (17.4 ± 5.4 ng/ml) with significant association between different hypertensive groups than in hypertensive controls (9.1 ± 0.75 ng/ml).

Greater degree of disability was observed in hypertensive stroke patients with raised serum NSE and hypertensive patients with mean serum NSE level of 22.9 ± 3.6 ng/ml and dyslipidemia had greater probability of neurological worsening as compared to those with mean serum NSE level of 12.7 ± 1.2 ng/ml. Serum NSE levels can serve as a peripheral indicator of neuronal damage and assist in the prediction of disability and clinical outcome in hypertensive cerebrovascular ischemic stroke patients.

Keywords Neuron specific enolase (NSE) · National Institute of Health Stroke Scale (NIHSS) · World Health Organization (WHO) · International Society of Hypertension (ISH)

Introduction

Cerebrovascular stroke is one of the most common devastating disorder, being the leading cause of disability and third major cause of death worldwide after cardiovascular diseases and cancer. Rapid detection and evaluation of patients presenting with cerebrovascular stroke is of great clinical relevance. Blood pressure is a major risk factor for cerebrovascular disease and the main modifiable risk factor for stroke. BP-lowering reduces the risk of stroke, deaths from stroke, and the burden of disability related to stroke [1]. Hypertension causes blood–brain barrier breakdown by mechanisms involving inflammation, oxidative stress, and vasoactive circulating molecules [2–5]. High blood pressure has been reported to predispose to development of white matter hyperintensities (WMH), lacunar infarction and microbleeds.

However management and lowering of blood pressure in cerebral ischemic stroke is still uncertain. Too aggressive

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BP—lowering in cerebral ischemic stroke may lead to extension of brain infarction in already threatened tissue of the ischemic penumbra, which may have poor autoregulatory capability whereas high BP can potentiate leaky ischemic blood vessels to be more susceptible to mediating brain edema, hemorrhagic transformation, and neurologic worsening [6].

At present only invasive and expensive techniques such as contrast-enhanced magnetic resonance imaging, CT-scan are available to test clinically BBB integrity, having their own limitations. Computed tomography (CT) is insufficiently sensitive to detect ischemic stroke, especially in the early phase and in cases of lacunar or posterior fossa infarction [7]. Magnetic resonance imaging (MRI) has greater sensitivity for detecting ischemic stroke but can still appear normal in the first hours [8] and is also not available in every emergency department.

NSE is a $\gamma\gamma$ -isoenzyme of the glycolytic enzyme enolase found mainly in cytoplasm of neurons and cells of neuroendocrine origin. When the plasma membrane is impaired functionally or structurally, NSE is released from damaged neurons [9–13].

As occurrence of brain damage is frequently associated with release of neuronal proteins into peripheral circulation, this study was undertaken to evaluate the utility of brain biomarker neuron specific enolase in predicting neurological worsening in hypertensive patients which can thus assist in management and formulation of prevention strategies in hypertensive stroke patients. *The objective of this work was to examine the status of serum NSE in hypertensive patients, and its possible implication as a marker of neurological outcome in such patients.*

Materials and Methods

The study was conducted by the Department of Biochemistry in collaboration with the Department of Neurology at Sri Aurobindo Institute of Medical Sciences, Indore, Madhya Pradesh, between January 2011 and July 2014. Informed consent was taken from the patients or nearest relatives before inclusion in the study. This work was approved by the Institute Ethics Committee, selection of cases was done from in-patient wards and ICU from the Department of Neurology and Department of Medicine, SAIMS Medical College, Indore. Detailed clinical history was taken from every patient and neurological, general, and systemic examination was carried out on all patients.

The subjects were divided into two groups: the first group consisted of 60 controls having essential hyperten-

sion coming to hospital because of regular checkup or headache but with no neurological disease. Study population included 80 hypertensive ischemic stroke patients diagnosed by a neurologist as per WHO definition [14] along with CT Scan/MRI suggestive of cerebrovascular stroke and differentiating from hemorrhagic stroke. All the volunteers were explained the purpose of the experiment, and a written consent was obtained from each of them. Patients with Transient ischemic attack, hemorrhagic stroke, major cardiac, renal and hepatic diseases, CNS tumors, recent head injury, and history of previous stroke were excluded from the study. Blood pressures were always measured with a calibrated sphygmomanometer, in the right arm, with subjects in the supine position. Hypertensive patients were classified into 4 groups as per 1999 WHO/ISH criteria 140–159/90–99; 160–179/100–109; $\geq 180/\geq 110$; $\geq 140/\leq 90$ [15].

Detailed neurological examination using the National Institute of Health Stroke Scale (NIHSS) [16] was performed and neurological disability was assessed by NIHSS at the time of admission (within 72 h from the onset of stroke) and on 7th day after admission. Depending on the disability score, cases were categorized into mild, moderate and severe disability. Early neurological worsening was diagnosed as an increase in National Institute of Health Stroke Score (NIHSS) by two or more points (or stroke-related death) between admission and day 7 [17] and who remained stable or improved in the same period was classified as “no worsening”.

10 ml venous blood sample was collected from all subjects in a plain serum separator tube. For patients with stroke, the venous blood samples were drawn within 72 h from the onset of symptoms. The samples were processed as per the laboratory protocol. The serum NSE samples were analyzed using an enzyme immunoassay manufactured by DRG Instruments GmbH, Germany based on the sandwich technique including the solid-phase monoclonal antibody raised against c-NSE. The sensitivity of the NSE assay is 1 ng/ml. Routine hematological and hemochemical analyses were performed (hemoglobin, hematocrit, leukocyte count, glucose, creatinine, uric acid, lipid profile, LFT).

Statistical Analysis

Data analysis was done by SPSS version 23. For normality test Shapiro–Wilk test was used. Non parametric Kruskal–Wallis and Mann–Whitney U test were applied to compare means in data not normally distributed. Correlation was done using Spearman correlation.

Results

The clinical and demographic characteristics of the patients with confirmed hypertensive ischemic cerebral stroke, high-risk controls are presented in Table 1.

Median and interquartile range for age of the patients was 61 ± 12 years, minimum age was 34 years and the maximum was 76 years. Out of 80 patients 48 were males and 32 were females. Mean Systolic BP in study group was 162 ± 13.92 mmHg and mean Diastolic BP was 100.74 ± 10.5 mmHg. 96% of patients in study group had essential hypertension of more than 5 years of duration and were on antihypertensive drugs. 41.3% had serum triglyceride >150 mg/dl and total cholesterol >200 mg/dl. Mean NSE in study group was 17.4 ± 5.4 ng/ml. In controls mean SBP was 156.5 ± 12.4 mmHg, mean DBP 97.2 ± 6.9 mmHg and mean NSE was 9.1 ± 0.75 ng/ml. The difference between mean NSE values in cases and controls was 8.3 ± 5.5 and was found to be statistically significant ($p < 0.05$).

From Table 2 significant association with NSE was observed between different groups of hypertensive stroke patients ($p < 0.05$). At the time of admission 25% of patients with SBP > 180 had NSE 22.2 ± 7.2 ng/ml and 8.0% had dyslipidemia; 31.3% with SBP between 160–179 had NSE 18.3 ± 5.0 ng/ml and 11.3% had dyslipidemia; 43.8% with SBP between 140–159 had NSE 15.7 ± 4.2 ng/ml and 21.3% had dyslipidemia. 20% Of patients with DBP < 90 had mean NSE 17.7 ± 4.4 ng/ml and 11.3% had dyslipidemia; 23.8% with DBP between

90–99 had NSE 14.0 ± 3.4 ng/ml and 10% had dyslipidemia; 18.8% with DBP between 100–109 had NSE 16.9 ± 5.2 ng/ml and 5% had dyslipidemia; 37.5% with DBP > 110 had NSE 18.1 ± 5.1 ng/ml and 15% had dyslipidemia. The difference in NSE means was found to be statistically significant in different hypertensive groups ($p < 0.05$) In controls having SBP > 160 mmHg mean NSE was ≥ 9.0 ng/ml.

In Table 3 patients with mild disability at the time of admission had mean SBP 160 ± 13.0 , DBP 100.4 ± 9.2 ; in moderate disability mean SBP 164.9 ± 13.7 , DBP 101.2 ± 11.3 ; in severe disability SBP 164.7 ± 16.1 , DBP 100.5 ± 12.4 . Distribution of SBP and DBP was found to be same across different categories of neurological disability and there was no statistically significant difference in means as determined by Kruskal–Wallis test (p value 0.372; and 0.679). 80% of patients with severe disability had dyslipidemia. Hypertensive stroke patients with higher serum NSE levels presented with greater degree of disability. Mean serum NSE in mild disability was 14.1 ± 3.5 ng/ml; moderate disability 19.4 ± 5.1 ; severe disability 24.8 ± 4.2 ng/ml and the difference was found to be statistically significant.

From Table 4 a significant association of neurological worsening was seen ($p < 0.05$) with Diastolic blood pressure, serum NSE and associated dyslipidemia in hypertensive patients. In this study hypertensive patients with mean serum NSE level of 22.9 ± 3.6 ng/ml and dyslipidemia had greater probability of neurological worsening as compared to those with mean serum NSE level of 12.7 ± 1.2 ng/ml. Spearman's rank correlation coefficient for SBP and NSE is 0.414 and for DBP and NSE is 0.371 showing a positive correlation between them which is statistically significant at 0.01 level (2-tailed).

Table 1 Demographic profile

Demographical variables	Subjects n = 140	
	HT cases n = 80	HT controls n = 60
<i>Age category (years)</i>		
<40	02 (2.5%)	10 (16.6%)
40–59	29 (36.2%)	35 (58.3%)
>60	49 (61.2%)	15 (25%)
<i>Gender</i>		
Female	32 (40%)	35 (58.3%)
Male	48 (60%)	25 (41.6%)
<i>Smokers</i>		
No	52 (65%)	40 (66.6%)
Yes	28 (35%)	20 (33.3%)
<i>Blood pressure (mmHg)</i>		
140–159/90–99	20 (25%)	34 (56.7%)
160–179/100–109	25 (31.2%)	17 (28.3%)
>180/>110	20 (25%)	04 (6.7%)
140/70–89	15 (18.7%)	05 (8.3%)

Discussion

Hypertension plays a role in numerous mechanisms of stroke. Many individuals with high blood pressure are unaware that they have it; many who are aware are untreated or undertreated. In previous studies a significant negative relationship was found between blood pressure decrease and improvement in cerebral blood flow as measured by single photon emission tomography after ischemic stroke in humans [18].

Physiologically, NSE is present only in negligible amounts in the peripheral blood (serum concentration is 8.7 ± 3.9 ng/ml (men 8.9 ± 3.9 ng/ml, women 8.3 ± 4.0 ng/ml) [19]. Raised NSE levels could reflect initial brain deterioration in a subset of hypertensive patients as compared to hypertensive asymptomatic patients. Subclinical cerebrovascular damage, development of white matter hyperintensities (WMH), lacunar infarction

Table 2 Association between SBP and DBP with NSE in cases

NSE (ng/ml)	SBP (mmHg) n/ % within SBP cat			<i>p</i> value	DBP (mmHg) n/ % within DBP cat				<i>p</i> value
	140–159	160–179	≥180		<90	90–99	100–109	≤110	
5–15	19 (54.3%)	10 (40%)	07 (35%)	0.00	06 (37.5%)	13 (68.4%)	08 (53.3%)	09 (30%)	0.002
15.1–25	16 (45.7%)	14 (56%)	03 (15%)		10 (62.5%)	06 (31.6%)	06 (40%)	11 (36.7%)	
>25	0 (0.00)	01 (04%)	10 (50%)		00 (0.00%)	00 (0.00%)	11 (36.7%)	10 (33.3%)	

Significance at $p < 0.05$ **Table 3** Association between disability and blood pressure, NSE, dyslipidemia

	Mild n/(% of total)	Moderate n/(% of total)	Severe n/(% of total)	Asymptotic significance (2-sided)
<i>SBP</i>				
140–159	16 (20%)	12 (15%)	07 (8.8%)	0.212
160–179	14 (17.5%)	09 (11.3%)	02 (2.5%)	
≥180	05 (6.3%)	09 (11.3%)	06 (7.5%)	
<i>DBP</i>				
<90	05 (6.3%)	07 (8.8%)	04 (05%)	0.210
90–99	11 (13.8%)	05 (6.3%)	03 (3.8%)	
100–109	10 (12.5%)	04 (05%)	01 (1.3%)	
≥110	09 (11.3%)	14 (17.5%)	07 (8.8%)	
<i>NSE</i>				
5–15	27 (33.8%)	09 (10%)	00 (0%)	0.000
15.1–25	08 (10%)	16 (20%)	09 (11.3%)	
>25	0 (0%)	05 (6.3%)	06 (7.5%)	
<i>Dyslipidemia</i>				
No	33 (44.3%)	11 (13.8%)	03 (3.8%)	0.000
Yes	02 (2.5%)	19 (23.8%)	12 (15%)	

Significance $p < 0.05$

and microbleeds have been reported to be more frequent in hypertensive subjects [20–22].

The degree of elevation of systolic and diastolic blood pressure is correlated with the risk of stroke. Systolic hypertension is accompanied by increased arterial stiffness and decreased compliance, and as a result diastolic blood pressure often decreases accounting for neurological worsening in subjects having isolated systolic hypertension in this study. Epidemiological studies have identified several risk factors for ischemic stroke, but high blood pressure is the most important of the known risk factors for all stroke [1].

Necrosis is currently believed to be the predominant form of neuronal death in focal ischemia and ischemic stroke in humans characterized morphologically by cell swelling and eventual rupture, which releases contents into the surroundings. Irreversible loss of energy-dependent cellular ion gradients causing cytotoxic edema causes neuronal death with reduction in cerebral blood flow below 10–15 mL/100 g per minute for more than a short period of

time [23]. Association of significantly raised serum NSE levels in hypertensive stroke patients as compared to hypertensive controls could be the result of cellular cytoskeletal breakdown due principally to energy failure of the cell and neurochemical expression of damaged brain tissue in this situation. Brain hypoperfusion and breakdown of the blood brain barrier, leading to decreased cerebral blood flow and consequent cerebral ischemia may lead to increased serum NSE levels. Raised NSE levels have been found to be associated with a higher severity of hypertension and of white matter lesions, suggesting the presence of silent brain damage in a subset of hypertensive patients [24]. High intraluminal pressure during hypertension leads to extensive alteration in endothelium and smooth muscle function in intracerebral arteries increasing permeability over the blood–brain barrier and local or multifocal brain oedema [25]. Elevated NSE levels in this study could point to neurochemical expression from damaged brain tissue in hypertensive patients.

Table 4 Association between neurological worsening and blood pressure, NSE and dyslipidemia after 7 days

	NW		Asymptotic significance (2-sided)
	No n/ % of total	Yes n/ % of total	
<i>SBP</i>			
140–159	20 (25%)	15 (18.8%)	0.262
160–179	11 (13.8%)	14 (17.5%)	
≥180	07 (8.8%)	13 (16.3%)	
<i>DBP</i>			
<90	06 (7.5%)	10 (12.5%)	0.015
90–99	14 (17.5%)	05 (6.3%)	
100–109	09 (11.3%)	06 (7.5%)	
≥110	09 (11.3%)	21 (26.3%)	
<i>NSE</i>			
5–15	36 (45%)	00 (00%)	0.000
15.1–25	02 (2.5%)	31 (38.8%)	
>25	00 (00%)	11 (13.8%)	
<i>Dyslipidemia</i>			
No	32 (40%)	06 (7.5%)	0.000
Yes	06 (7.5%)	27 (33.8%)	

Significance $p < 0.05$

Hypertensive patients with raised serum NSE (≥ 15 ng/ml) and with dyslipidemia were found to have greater degree of disability in our study as compared to hypertensive patients with serum NSE (≤ 15 ng/ml). Serum NSE levels thus could be a reflection of clinical severity in hypertensive stroke patients. Serum NSE concentration has been reported to be a useful biomarker to predict subclinical brain damage and future vascular events related with the CNS in hypertension [26, 27].

Neurological deterioration was associated with higher diastolic pressure though a significant association was noted between neurological disability and higher SBP and DBP. Higher DBP associated with poor neurological outcome might be due to hemorrhagic transformation. Many observational studies have shown a J-shaped relationship between blood pressure and outcome, with the best outcome for a systolic blood pressure around 150 mmHg [28–30]. This discrepancy is probably accounted for by different type (cardioembolic, atherothrombotic, or lacunar), site, and severity of stroke.

Conclusion

As relationship between raised blood pressure and neurological worsening in ischemic stroke is still controversial, serum bio marker NSE for neuronal damage has been implicated for estimating the extent of cerebral injury in hypertensive ischemic cerebrovascular stroke along with

CT Scan/MRI. The use of serum NSE as early and quantitative markers could prove important as a potential tool in the hands of clinicians for the detection and management of initial brain deterioration in hypertensive patients.

High systolic and diastolic blood pressure are associated with increased disability and neurological deterioration as evidenced by increased levels of serum NSE in these patients. Although our study points positive association between elevated blood pressure, serum NSE and neurological outcome, it is limited by types of ischemic stroke which needs to be further evaluated.

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