**Abstract**

Objectives: The aim of this study is to see the role of serum NSE (Neuron Specific Enolase) as a neuronal damage marker and in differentiating the side of brain lesion in acute ischemic stroke patients.

Methods: A total of 35 acute ischemic stroke patients (clinically and radiologically confirmed) irrespective of age and sex, admitted in Emergency Unit, Medicine Department within 24 hours of stroke onset were included in this study. Plain CT scan or MRI brain was done for all these patients on admission and after 48 hours to confirm the diagnosis and the side of the lesion in brain. Serum NSE was estimated by using NSE Human ELISA Kit, in the Department of Physiology.

Results: In this study, serum NSE bears a positive significant correlation to the infarct volume in brain (r=0.783, p<0.001) and to the National Institute of Health Stroke Scale (NIHSS) (r=0.538, p=0.001). However, there is no significance difference between the serum NSE in right hemispheric brain lesion compared to left hemispheric brain lesion (p=0.596).

Conclusions: Serum NSE within 24 hours of stroke onset can reflect the volume of brain lesion and severity of acute stroke but cannot differentiate the side of brain lesion in these patients.

Keywords: Ischemic, NSE, CT, NIHSS

**1. Introduction**

Stroke can be defined by the neurological deficit of sudden onset that is due to a vascular cause of focal origin.[1] Ischemic stroke, a major subtype of acute stroke occurs due to loss of blood supply to a part of brain which initiates a ischemic cascade leading due to free radical production and damage to endothelial lining. Atherosclerosis or embolic infaracts can break off the circulation and may block a vasculature supplying a part of brain causing neuro-ischemic cascade. These will lead to neuronal injury resulting in loss of structural integrity of brain tissue and blood vessels. At present, diagnosis of stroke is mostly based on CT scan (Computer Tomography) or MRI (Magnetic Resonance Imaging).[2] Neuron-specific Enolase(NSE) is the neuronal form of the intracytoplasmic glycolytic enzyme enolase. The γγ isoform is found in neurons, as well as in cells with neuroendocrine differentiation. This dimeric enzyme has a molecular weight of 78 kDa and catalyzes the interconversion of Phosphoglcerate and Phosphoenolpyruvate during the glycolysis. Since NSE is not secreted physiologically, an increase in serum and cerebrospinal fluid (CSF) concentrations is considered to be a marker for neuronal cell damage.[3] NSE is found mainly in the cytoplasm of neurons and cells of neuroendocrine origin. It has also been found in erythrocytes and platelets, but in smaller concentrations. Elevated concentrations of NSE have been noted in ischemic and hemorrhagic stroke, head injuries and NSE estimation can reflect
the state of nervous tissue damage. The level of Neuron Specific Enolase increases in blood circulation following acute stroke as it is not used anymore in damaged neurons.

To the best of my knowledge, literature regarding the role of serum NSE in differentiating the side of brain lesion in acute ischemic stroke patients are lacking in Indian as well as in international literature point of view. Hence, the study had been conducted to see the significance of serum NSE as a marker of neuronal damage and in differentiating the side of brain lesion in acute ischemic stroke patients in a tertiary-care hospital of North India.

2. Material and methods

The study had been conducted in collaboration with the Department of Physiology and Department of Medicine after getting clearance from the Institutional Ethical Committee from March 2013 to January 2015. A total of 35 acute ischemic stroke patients (clinically and radiologically confirmed) irrespective of age and sex, admitted in Emergency Unit of the Medicine Department within 24 hours (<24 hours) of symptom onset of stroke were included in this study. Those patients who had a history of suffering from any neurological disease like neuro-blastoma, neurodegenerative disorders like dementia, epileptic seizure, encephalopathy, chronic sequence of stroke, multiple stroke attacks, traumatic brain injury, hemolytic anemia, hepatic failure, end stage renal disease, some carcinomas like small cell lung carcinoma, Medullary thyroid cancer, Carcinoid Tumor, Islet cell tumours of pancreas and melanoma etc were excluded from the study.

A pre designed semi-structural proforma, for each of the acute ischemic stroke patients was maintained where in brief clinical information including particulars of the patient such as chief complaints, family, personal, dietary, past history etc were recorded systematically. Proper general physical examination and systemic examination for all these patients were done and recorded in the proforma. Informed consent from the patient/patient party was also obtained before including them in the study. 5 ml of venous blood samples had been collected under aseptic condition from all the 35 acute ischemic stroke patients on admission (within 24 hours of onset of acute stroke) in a plain vial so as to allow the blood to coagulate. Coagulated collected samples had been ultra centrifuged so as to separate serum. The acute ischemic stroke patients were subjected to plain CT scan brain or MRI (depending upon the patient’s affordability) on admission in order to confirm the diagnosis and to estimate volume of lesion in brain. Beside a second CT scan (Model 16 slice Brivo 385) was done for these patients after 48 hours to confirm the diagnosis and to measure the volume of lesion more accurately. Volume of brain lesion was estimated from the film by using the formulae axbxc/2.[6] Serum NSE for the ischemic stroke patients was estimated by using NSE Human ELISA(Enzyme Linked Immunosorbent Assay) kit, Model-34239374 marketed by MyBiosource, Inc, USA which had been read by Microplate Reader (ELISA reader) Model No-iMark, 11915, Made in Japan(Marketed by Biorad Labs) in the Department of Physiology.

2.1 Ethical Approval

This study was conducted only after getting clearance from the Institutional Ethical Committee of King Georges Medical University, Lucknow (Ref code: 67th ECM II-B/P17). Beside, written informed consent was obtained from all the patient/patient party before including them in the study.

2.2 Statistical Methods

Data so collected were checked for consistency and were analyzed using the Statistical Package for Social Sciences (SPSS), version 16 (SPSS Inc, Chicago, IL, USA). Statistical methods like Spearman correlation coefficient and Mann Whitney Test etc were applied wherever found appropriate.

3. Results

The present study is based on the primary data of 35 acute ischemic stroke patients. In 35 acute ischemic stroke patients, serum NSE bears a positive significant correlation to the volume of infarction in brain (Spearman Correlation Coefficient \( r = 0.783, p < 0.001 \)) (table 1). Serum NSE also bears positive significant correlation to the NIHSS \( (r=0.538, p=0.001) \) (table 2). Beside, serum NSE also shows negative significant correlation to the Glasgow Coma Scale in these patients \( (r= -0.444, p=0.007) \) (table 3). In this study, majority (54.28%) of the acute ischemic stroke patients had left hemispheric brain lesion and remaining 45.72% of the patients had right sided brain lesion. However, there is no significance difference between the serum NSE in left sided brain lesion compared to the right sided brain lesion (p= 0.596) (table 4).
Table 1: Correlation of Serum Neuron Specific Enolase(NSE) to the volume of infarct in acute ischemic stroke patients

| Cases                        | Parameters               | Median±Standard Deviation (SD) | Correlation Coefficients (R) | P value |
|------------------------------|--------------------------|--------------------------------|------------------------------|---------|
| Acute ischemic stroke patients (n=35) | Volume of Infarction (cc) | 90.4±78.13 cc                  | Spearman Correlation (vol.=nonparametric) r=0.783 | P< 0.001 |
|                              | Serum NSE (ng/ml)        | 19.81±13.27 ng/ml              |                              |         |

Table 2: Correlation of Serum Neuron Specific Enolase (NSE) to NIHSS (National institute of health stroke scale) in acute ischemic stroke patients

| Cases                        | Parameters | Median± SD              | Correlation Coefficients (r) | P value |
|------------------------------|------------|-------------------------|------------------------------|---------|
| Acute ischemic stroke patients (n=35) | Serum NSE (ng/ml) | 19.81±13.27 ng/ml      | Spearman Correlation Coefficient r = 0.538 | P=0.001 |
|                              | NIHSS      | 17.4±8.01               |                              |         |

Table 3: Correlation of Serum Neuron Specific Enolase (NSE) to Glasgow coma scale in acute ischemic stroke patients

| Cases                        | Parameters               | Median± SD              | correlation coefficients (r) | P value |
|------------------------------|--------------------------|-------------------------|------------------------------|---------|
| Acute ischemic stroke patients (n=35) | Serum NSE (ng/ml)        | 19.81±13.27 ng/ml      | Spearman correlation r = -0.444 | P=0.007 |
|                              | Glasgow-Coma Scale(GCS)  | 10±4.11                 |                              |         |

Table 4: Comparison between the serum Neuron specific enolase(NSE) level in left sided and right sided lesion in acute stroke patients

| Cases                        | Parameters              | Median±SD               | Mann-Whitney Test               | p value |
|------------------------------|-------------------------|-------------------------|--------------------------------|---------|
| Acute ischemic stroke patients (n=35) | Left sided stroke     | 19.45±14.35             | Mann Whitney U value =136.00 Wilcoxon= 326 | p= 0.596 |
|                              | Right sided stroke     | 20.23±12.32             |                               |         |

5. Discussion

In the present study, serum NSE within 24 hours of ischemic stroke onset bears a positive significant correlation to the volume of infarction in acute ischemic stroke patients (Spearman Correlation Coefficient r=0.783, p<0.001). In another study, a group of authors also reported high correlation between the serum NSE to the volume of lesion within 24 hours of acute ischemic stroke onset(r=0.62, p<0.001).[7] A group of Indian authors also reported a positive significant correlation between concentration of NSE on day 1 and infarct volume as determined by CT scan in acute ischemic stroke patients(r=0.955, p<0.001).[8] In another study also, a significant positive correlation of serum NSE level to the CT scan volume of lesion( r=+0.993; p<0.001) has been reported in acute ischemic stroke patients within 24-48 hours of stroke onset.[9] The positive significant correlation between the serum NSE and volume of ischemia suggest that serum NSE can reflect the volume of neuronal damage in acute ischemic stroke. In the present study, serum NSE shows inverse significant correlation to the Glasgow Coma Scale (GCS) in acute ischemic stroke patients (r=-0.444, p=0.007). A strong negative correlation was found between GCS at presentation and concentration of NSE on day 1 of admission (r=−0.806, p<0.001) as reported by a group of Indian authors.[8] However, no studies have been reported within 24 hours of acute ischemic stroke onset which shows the correlation of the Glasgow coma scale to the serum NSE level in acute ischemic stroke patients. The negative correlation is because of the inverse relationship between the level of consciousness and the serum NSE. In this study, a positive significant correlation has been observed between the NIHSS and serum NSE within 24 hours of ischemic stroke (r=0.538, p=0.001). Another study also reported a positive significant correlation between serum NSE and NIHSS within 24 hours of acute ischemic stroke (r=0.42, p=0.002).[7] This significant correlation means that serum NSE can reflect the severity of stroke and neurological deficit in acute ischemic stroke patients. However, in this study, it has been observed that there is no significant difference between the serum NSE in left vs right sided stroke in acute ischemic stroke patients which means that serum NSE cannot differentiate the side
of lesion in brain. No such study has been reported yet to the best of our knowledge which focuses on the role of serum NSE in differentiating the side of the lesion in brain in acute ischemic stroke patients.

6. Conclusion
NSE is an important marker of neuronal damage in acute ischemic stroke. Serum NSE within 24 hours of stroke can reflect the volume of ischemia in acute ischemic stroke patients. Serum NSE within 24 hours can be correlated to NIHSS (severity of stroke) on admission in acute ischemic stroke. However, serum NSE cannot differentiate the side of brain lesion in acute ischemic stroke patients.

Acknowledgement
We are grateful to hospital staffs and technical persons who have given their valuable help and support during the study.

Findings
This study was supported by the Contingency Grant and Fellowship from ICMR (Indian council of Medical Research) which has given financial support to the study.

References
[1] Smith WS, English JD, Johnson SC. Cerebrovascular Diseases. In: Favei AS, Bravnauld E, Kasper DL, Hsusor SL, Longo DL, Joneson J, et al, editors. Harrison’s Principles of Internal Medicine. 17th ed. USA: McGraw Hills; 2008.p.2513-35.
[2] Allen CMC, Lueck CJ. Cerebrovascular Diseases In: Haslett Ch, Chilvers ER, Boon NA, Colledge NR, editors. Davidson’s principles and practice of Medicine. 19th ed. India: Churchill Livingston; 2002.p.1159-68.
[3] Brea D, Sobrino T, Blanco M, Cristobo I, Rodri R, Yan MR et al. Temporal profile and clinical significance of serum neuron-specific enolase and S100 in ischemic and hemorrhagic stroke. Clin Chem Lab Med 2009; 47(12):1513–8.
[4] Magorzata KO, Ryszard T, Andrzej F. Neuron-specific enolase and S-100B protein as predictors of outcome in ischaemic stroke. Neurologia I Neurochirurgia Polska 2010; 44(5): 459–63.
[5] Wardyani NS, Nurimaba N, Kurniani N. Correlation of Serum Neuron-Specific Enolase with severity and functional outcome in acute atherothrombotic infarction stroke patients. MKB 2010; 42(2):62-8.
[6] Sims JR, Gharai LR, Schaefer PW, Vangel M, Rosenthal ES, Lev MH et al. ABC/2 for rapid clinical estimate of infarct, perfusion, and mismatch volumes. Neurol 2009; 72(24): 2104–2110.
[7] Oh SH, Lee JG, Na SJ, Park JH, Choi YC, Kim WJ. Prediction of early clinical severity and extent of neuronal damage in anterior–circulation infarction from the initial serum Neuron Specific Enolase level. Arch Neurol 2003; 60:37-41.
[8] Zaheer S, Beg M, Rizvi I, Islam N, Ullah E, Akhtar N. Correlation between serum neuron specific enolase and functional neurological outcome in patients of acute ischemic stroke. Ann Indian Acad Neurol 2013 Oct-Dec; 16(4): 504–5085.
[9] Lukas DL, Retnowati E, Islam S. Role of serum Neuron Specific Enolase(NSE) to differentiate ischemic stroke from hemorrhagic stroke and its correlation with brain damage volume. Folia Medica Indonesiana 2007 Oct–Dec; 43(4): 230-4.