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Study of Neuron-Specific Enolase as Potential Biomarker for Assessing the Severity and Outcome in Patients with Cerebrovascular Accidents

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Study of Neuron–Specific Enolase as Potential Biomarker for Assessing the Severity and Outcome in Patients with Cerebrovascular Accidents

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Aim: The goal of present study was to measure diagnostic value of serum neuron specific enolase in various types of cerebrovascular accident as well as to evaluate the clinical performance of neuron specific enolase in early diagnosis of cerebrovascular accident.

Methods: A diagnostic case control study was conducted on 60 patients were admitted within 72 hours of onset of stroke in the department of neurology and department of medicine of PDVVPF's Medical College and hospital Ahmednagar and 60 healthy age and sex matched volunteers formed the control group. Serum neuron specific enolase level was estimated by commercially available quantitative enzyme linked immune sorbent assay (ELISA) kit which based on biotin double antibody sandwich technology.

Statistical analysis used: The student t test was used to compared patients and control. Receiver operating characteristic curve for neuron specific enolase was established to determined cut-off point. The sensitivity and specificity of neuron specific enolase for detection of cerebrovascular accident were analyzed.

Results: serum neuron specific enolase ($p<0.05$) concentrations was significantly higher in cerebrovascular accident than healthy controls. Sensitivity, specificity, positive predictive value and negative predictive value of neuron specific enolase for detection of cerebrovascular accident were 87.10%, 95.00%, 92.74% and 87.69%. The area under the receiver operating characteristic curve of neuron specific enolase in cerebrovascular accident was 0.84.

Conclusion: Present study shows that, assessment of serum neuron specific enolase level may be a useful Marker for severity in cerebrovascular accident like ischemic stroke and hemorrhagic stroke and it may be well correlated with neurological disability and short term functional outcomes.

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I. Introduction

Stroke or cerebrovascular accident (CVA) is the third leading cause of death after cardiovascular disease and cancer (1). In fact it is a leading cause of morbidity and mortality in major industrial countries. Approximately 20 million people each year are suffer from stroke (2).

India will face enormous socio-economic burden to meet the cosis of rehabilitation of stroke victims. Because the population, it is now surviving through peak years (age 55-65years) of occurrence of stroke (3).

The two major mechanisms causing brain damage in stroke are ischemia and hemorrhage. The effects of ischemia are fairly rapid because the brain does not store glucose, the chief energy substrate and is incapable of anaerobic metabolism. Intracerebral hemorrhage originates from deep penetrating vessels and causes injury to brain tissue by disrupting connecting pathways and causing localized pressure injury.4

The diagnostic and management of CVA is limited by lack of rapid diagnostic assay for use in emergency setting. In recent years, neurobiochemical markers of brain damage have gained particular attention in the identification of stroke patients with an adverse neurological outcome. The serum Neuron – Specific Enolase (NSE) level is one of these markers which can provide early information about neuronal damage. 5

Neuron – specific Enolase (NSE; EC 4.2.1.11) is an acidic soluble protein which functions as glycolytic isoenzyme. It is a 78 kD gamma homodimer and represents the dominant enolase isoenzyme which found in the cytoplasm of neurons and cells with neuroendocrine differentiation. 6

The measurement of NSE concentration in serum and cerebrospinal fluid (CSF) following cerebral

ischemia and traumatic head injury provides a reliable laboratory indicator of the degree of brain cell damage and may allow for early prediction of outcome. 7 An increased NSE concentration in blood has been reported in patients with small cell lung cancer, neuroblastoma and neurological disorders. 7, 8

Thus, in view of above information and several risk of complication, it is worthwhile to study the various biomarkers in CVA. Very few studies have been reported from serum NSE testing and its application in Indian context. The initial aim of our study was to measure the serum NSE levels in various types of CVA within in 72 hours of admission. Furthermore, the remarkable intention of present research was to evaluate the clinical performance of NSE in early diagnosis of CVA and monitoring tool for early prediction of ischemic stroke.

II. Material and Method

The present diagnostic case-control study was conducted at department of Biochemistry in PDVVPF's Medical College Ahmednagar with all participants providing informed consent and utmost care was taken during experimental procedure according to the declaration of Helsinki 1975.

a) Patients

Total 60 patients between age group 21 to 75 years admitted in the IPD wards of department of neurology and department of medicine were taken for the study. Data included history, clinical examination with laboratory investigation to exclude any other systemic or local disease that may affect the parameters examined in this study.

b) Control subjects

60 healthy age and sex matched individuals who didn't have any evidence of CVA as per clinical examination were taken as control subjects.

c) Inclusion criteria

Adult stroke (age> 21years) and within 72 hours of admission.

d) Exclusion criteria

CNS infection, Stroke more than 72 hours, and Peripartum stroke. All selected patients also subjected to the following protocol,

- Detailed neurological examination using the national institutes of health stroke scale,
- Computerized Tomography (CT) scan within 12 hours of admission to exclude patients with stroke mimic.

Approximately 5 ml blood was collected by venipuncture from anticubital vein of the forearm of each subject in plain vaccutainer (yucca diagnostic) under aseptic conditions within 72 hrs after admission and centrifuged for serum collection. Serum was stored at 20^0 C until assay was run to evaluate. All samples were thawed and analyzed in a single series.

III. Method

1) Determination of serum NSE: Serum NSE was measured with commercially available quantitative enzyme linked immune sorbent assay (ELISA) kit which based on biotin double antibody sandwich technology. Add serum containing NSE to well that is pre- coated with NSE monoclonal antibody and then incubate. After incubation, add NSE antibodies labeled with biotin to unite with streptavidin- HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, then add chromomegnic reagent A and B. colour change blue to yellow with effect of acid which positively correlated with concentration of human NSE. 9, 10

Statistical analysis: The statistical analysis was carried out by using the SYSTAT software package for window version 12. The students "t" test was applied for the statistical analysis and the results were expressed in mean± Standard Deviation (mean ±SD).p values $p<0.05$ for NSE were considered to be statistically significant. The Receiver Operating Characteristic (ROC) curve analysis and the area under the curve were performed for determination of diagnostic performance of serum NSE in the all patients included in the study. The optimum cutoff values for determination of serum NSE were selected from ROC analysis. This optimum cutoff was used to dichotomously classify positive or negative serum NSE level, and used for calculating of diagnostic sensitivity and specificity.

IV. Results

Baseline demographic and clinical characterization of the patients and healthy controls groups are given in table-1 there were no significant differences between the groups in age, gender.

Table 1 : Baseline characteristics of all subjects

Variables	Controls (n=60)	CVA	
		Ischemic stroke (n=32)	Hemorrhagic stroke (n= 28)
Age in years	40.1± 12.34	41.3±14.06	43.1± 13.01
Gender (Men/Women)	28/30	19/13	11/27
Systolic blood pressure (mmHg)	110 ± 15.03	119.15± 30.11	120.07 ± 35.16
Diastolic blood Pressure (mmHg)	75.62 ± 5.04	81.42± 14.28	79.53 ± 16.02
Cigarette Smokers (n)	------	07	15
Tobacco Chewing(n)	------	26	19
Atrial fibrillation (n)	07	06	04
DM (n)	9	12	8
Serum NSE (ng/ml)	14.55± 12.41	43.63± 13.41*	45.63± 15.89*

Values were expressed in mean with Standard Deviation (mean±SD),
**Statistically highly significant,(p<0.001)*
n =numbers

As shown in table-1, Serum neuron specific enolase levels were increased significantly ($p<0.05$) in the ischemic stroke Group (43.63±13.41) and hemorrhagic stroke (45.63±15.89) as compared to controls group (14.55±12.64).

The performance of Serum NSE for diagnosis of ischemic stroke and hemorrhagic stroke is presented in table- 2. Sensitivity, specificity, positive predictive value and negative predictive value were 84.38%, 95.00%, 90.00% and 91.94% respectively in Ischemic stroke. Similarly, in hemorrhagic stroke, Sensitivity, specificity, positive predictive value and negative predictive value were 89.29%, 95.00%, 89.29% and 95.00%.

Table 2 : Diagnostic performance of Serum NSE for detection of CVA

Types of CVA	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
Ischemic Stroke	84.38	95.00	90.00	91.94
Hemorrhagic stroke	89.29	95.00	89.29	95.00

Figure-2. shows a scatter plot distribution of the results of serum NSE in controls and CVA groups. The optimum diagnostic cut off point maximizing the sensitivity and specificity was determined to be 40 ng/ml with a sensitivity of 87.10 % and specificity 95%, the area under curve for NSE was 0.84

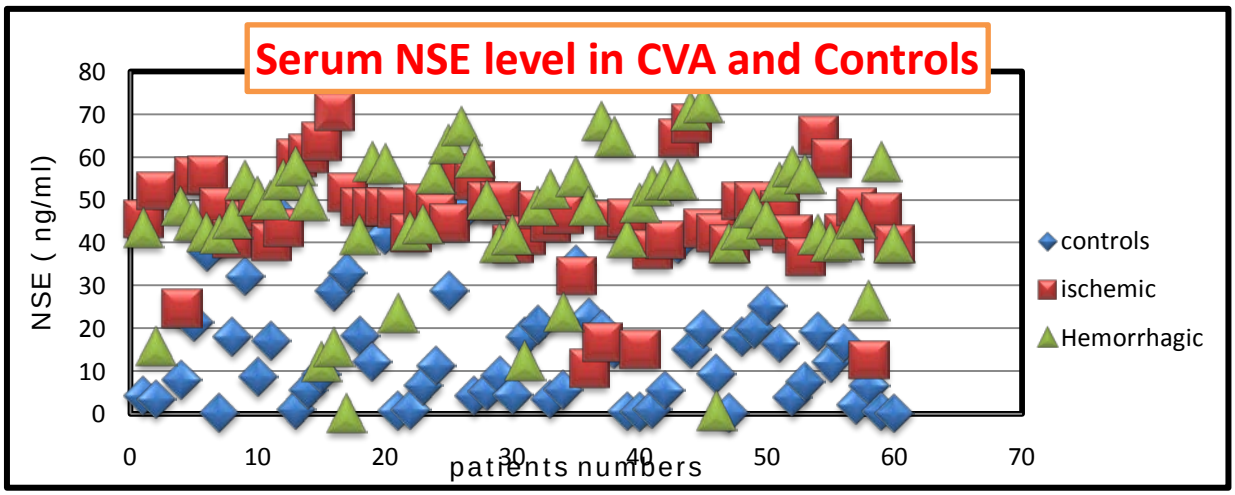

Figure 1 : Scatter plot distribution of the results of serum NSE in CVA and controls

V. Discussion

Stroke causes a vast amount of death and disability throughout the world. It is important to have sufficiently sensitive marker for brain damage that can be determined in blood instead of cerebrospinal fluid because blood samples can be taken more frequently and more independent of raised intracranial pressure than cerebrospinal samples. 11 Evaluation of enzyme level in Cerebrospinal fluid or serum has evoked keen interest as a simple, economical, reliable and easily available method for the evaluation of severity, course and prognosis and to some extent in the differential diagnosis of various types of CVA.Ischemia causes a cascade of event that eventually leads to neuronal damage and cell death. 12 NSE is the predominant enolase found in neural tissue, and the structural characteristics of this enolase allow for greater stability in high chloride concentrations compared with enolase in other organ system. 7

In the current study, Serum neuron specific enolase levels were increased significantly ($p<0.05$) in the ischemic stroke group and hemorrhagic stroke as compared to controls group. Our results are strongly supported to previous reports. 5,11,13, Increased NSE level in CVA may be due to cute CNS such as cerebral infarction, hypoxia, trauma and seizure the blood – brain barrier is altered and astroglial disintegration results in leakage of NSE into the serum and cerebrospinal fluid.13

Schaarschmidt H et. al. research where they have studied the NSE in relation to the severity CVA . They verified that, plasma NSE level is seen as a relevant parameter for assessing the prognosis of cerebral ischemia. Additionally it may prove to be a useful tool for monitoring space occupying brain infarctions and intracerebral hemorrhage and therefore may contribute to improved therapeutic management of severe cerebrovascular diseases. 11

Numbers of researchers have focused on the study of NSE in various types of CVA. Aparna Pandey et al have showed that initial serum NSE level may be a useful marker for severity in acute ischemic stroke and it may be well correlated with neurological disability and short term functional outcomes. They also suggested that, serum NSE may be used as an indicator of outcome in cerebral infarction patients. 14

Natheer H. Ravi and Karim M. Aantiyah have demonstrated that, salivary NSE alone or in combination with serum can be used as valuable diagnostic for measurement of neuronal damage in patients with stroke and stroke related diseases. According to them, in ischemic stroke, the integrity of blood- brain barrier is disrupted to various degrees in these patients and leakage of this enzyme outside the CNS can be seen in salivary secretion. 13 Takaaki kirino et al have documented that, NSE as a reliable enzymatic indicator of axon injury, regeneration and in particular of target innervations and reinnervation. 6

In cross- sectional comparative study, Diwi L Lukas et al have examined and compared serum NSE level in ischemic and hemorrhagic stroke patients according to lesion volume and also analyzed correlation between serum NSE level and lesion volume in CT scan as gold standard. According to them, serum NSE level in acute stroke patients (24-48 hours) after onset can be used to estimate the extent of brain damage (lesion volume) but it cannot be used to differentiate the type of stroke. 15

In present study, furthermore diagnostic performance of Serum NSE for diagnosis of ischemic stroke and hemorrhagic stroke were analyzed. The optimum diagnostic cut off point maximizing the sensitivity and specificity was determined to be 40 ng/ml with a sensitivity of 87.10 % and specificity 95% the area under ROC curve for NSE was 0.84. Our results are completely conformity with Hill et al study. They found that in a single examination, NSE had sensitivity of 89%. They also suggested that, in the future the examination of neurobiochemical marker panel like serum NSE, Myelin basic protein, Protein S-100 B etc can be used not only to differentiate the type of stroke but also to differentiate the subtype of acute stroke. 16 In addition, Natheer H Rawi and Karim M Atiyah have accomplished the diagnostic performance of NSE in patients with ischemic stroke and stroke prone patients. According to their result, the area under the ROC curve for serum NSE was significantly higher (0.960) compared to salivary NSE (0.825). The optimum cut-off value for serum NSE showing the highest diagnostic accurancy (90%) was $\geq$ 13.1µg/L. This cut-off threshold showed optimum specificity (100%) and reasonable sensitivity (85%).

VI. Conclusion

Our finding indicate that, serum NSE as brain biomarker in CVA had elevated compared to controls which provides insight into the pathophysiologic mechanism of brain injury. Serum NSE in CVA patients after onset (<72hours) may be used to estimate the extent of neuronal damage and can be reliable parameter for weigh up CVA.

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