Correlation between serum neuron specific enolase and functional neurological outcome in patients of acute ischemic stroke

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Abstract

Context: The use of biomarkers to predict stroke prognosis is gaining particular attention nowadays. Neuron specific enolase (NSE), which is a dimeric isoenzyme of the glycolytic enzyme enolase and is found mainly in the neurons is one such biomarker. **Aims:** This study was carried out on patients of acute ischemic stroke with the aims to determine the correlation between NSE levels on the day of admission with infarct volume, stroke severity, and functional neurological outcome on day 30. **Materials and Methods:** Seventy five patients of acute ischemic stroke admitted in the Department of Medicine were included in the study. Levels of NSE were determined on day 1 using the human NSE ELISA kit (Alpha Diagnostic International Texas 78244, USA). Volume of infarct was measured by computed tomography (CT) scan using the preinstalled software Syngo (version A40A) of Siemen's medical solutions (Forchheim, Germany). Stroke severity at admission was assessed using Glasgow coma scale (GCS) and functional neurological outcome was assessed using modified Rankin scale (mRS) on day 30. **Statistical Analysis Used:** Statistical analysis was performed using the SPSS software for windows version 15.0 (SPSS). **Results:** A positive correlation was found between concentration of NSE on day 1 and infarct volume determined by CT scan ($r = 0.955$, $P < 0.001$). A strong negative correlation was found between GCS at presentation and concentration of NSE on day 1 ($r = -0.806$, $P < 0.001$). There was a positive correlation between NSE levels at day 1 and functional neurological outcome assessed by mRS at day 30 ($r = 0.744$, $P < 0.001$). **Conclusions:** Serum levels of NSE in first few days of ischemic stroke can serve as a useful marker to predict stroke severity and early functional outcome. However, larger studies with serial estimation of NSE are needed to establish these observations more firmly.

Key Words

Glasgow coma scale, infarct volume, ischemic stroke, modified Rankin scale, neuron specific enolase

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Introduction

The use of biomarkers in diagnosis of stroke, and as predictors of stroke severity and prognosis is gaining particular attention in the recent times.[1] Neuron specific enolase (NSE) is one such biomarker; it is a dimeric isoenzyme of the glycolytic enzyme enolase and is found mainly in the neurons and cells of the neuroendocrine system.[2] NSE had gained attention recently as an auxiliary test in diagnosis of small cell carcinoma of lung,[3] neuroendocrine tumors,[4] and Creutzfeldt-Jakob disease.[5] Serum NSE levels were also found useful in predicting poor outcome in comatose patients after cardiopulmonary resuscitation for cardiac arrest.[6] Various studies have shown a positive correlation between NSE levels and infarct volume in patients of acute ischemic stroke,[1,7-10] whereas some studies have failed to demonstrate such relationship between NSE levels and infarct volumes.[11] Studies have also pointed out that there is a significant correlation between NSE levels and stroke severity on admission.[1,2,7,8] On the other hand, few investigators have found no such relationship between NSE levels and stroke severity at admission.[12] The ability of NSE levels to predict functional neurological outcome in stroke patients is also a matter of recent interest with some studies suggesting that NSE is useful in predicting functional outcome,[1,7-9,12] while the other studies suggesting otherwise.[10] In view of contradictory findings of these studies we conducted this study on patients of acute ischemic stroke with the aims of determining *(1)* the correlation between NSE levels at admission and infarct volume. *(2)* Correlation between NSE levels at admission and stroke severity. *(3)* Correlation between NSE levels at admission and early functional neurological outcome.

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Materials and Methods

Seventy five patients of acute ischemic stroke admitted in the Department of Medicine from December 2011 to November 2012 were included in the study. 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. Furthermore, excluded from the study were the patients who died within 1 month of stroke onset (As functional neurological outcome was assessed at day 30). Informed consent was taken from the patients or nearest relatives before inclusion in the study. The study was approved by local ethical committee. Detailed clinical history was taken from every patient and neurological, general, and systemic examination was carried out on all patients.

Stroke severity at admission was measured using the Glasgow coma scale (GCS).[13] GCS was measured and recorded as soon as patient reported to hospital.

Blood samples to determine NSE levels were collected from every patient on the day of admission (day 1), the serum was separated by centrifugation, and if the sera could not be immediately assayed it was stored at −20°C. NSE levels were measured by ELISA method using the human NSE ELISA kit (Alpha Diagnostic International Texas 78244, USA) as per manufacturer's instructions. NSE assay was carried out in the Department of Biochemistry and the performing biochemist was blinded to clinical and radiological characteristics of the patients.

Non-contrast computed tomography (CT) scan of the head was performed on the day of admission (day 1) to make the diagnosis of ischemic stroke, CT scan was then repeated on day 3 to measure the volume of infarction. Infarct volume was measured by a radiologist using pre-installed software Syngo (version A40A) of Siemen's medical solutions (Forchheim, Germany). The radiologist who measured infarct volume was blinded to clinical characteristics and NSE levels of the patients.

Functional neurological outcome was assessed on day 30 using the modified Rankin scale (mRS).[14]

Statistical analysis

Statistical analysis was performed using the SPSS software of windows version 15.0 (SPSS). Categorical variables were expressed as percentages and continuous variables were expressed as means ± standard deviations. All $P < 0.05$ were taken as significant. Independent-samples t test and one way ANOVA were used to compare means between the groups. Correlation between NSE levels, infarct volume, GCS and mRS were assessed using the bivariate correlation analysis. Multiple linear regression analysis was carried out to look for variables independently associated with outcome measure.

Results

Baseline clinical characteristics, vascular risk factors, neuroimaging findings, and neurological outcome measures of 75 patients with acute ischemic stroke are summarized in Table 1.

Mean age of the patients was 61.29 ± 12.57 years, minimum age was 35 years and the maximum was 86 years. Out of 75 patients 47 were males and 28 were females. Hypertension was present in 46 (61.3%) patients, diabetes mellitus was found in 34 (45.3%), history of tobacco smoking was found in 42 (56%), dyslipidemia was seen in 23 (30.67%).

Minimum infarct volume measured by CT scan was 7.72 ml; maximum infarct volume was 167.69 ml. Mean infarct volume was 53.88 ± 42.92.

Concentration of NSE was measured on day 1, minimum NSE concentration was 15.68 ng/l and maximum NSE concentration was 198.42 ng/l with a mean value of 64.36 ± 49.71 ng/l.

GCS was used to assess stroke severity at admission, mean GCS was 7.88 ± 3.28. mRS was used to assess neurological outcome at day 30, minimum mRS was 1 and the maximum was 6 with a mean of 3.96 ± 1.50.

Correlation of NSE with infarct volume

A positive correlation was found between concentration of NSE on day 1 and infarct volume determined by CT scan ($r = 0.955$, $P < 0.001$) [Figure 1]. For the purpose of this study, infarct volumes were arbitrarily classified into three groups, group 1 represented small infarcts of volume ranging from 0 ml to 10 ml, group 2 represented infarcts of intermediate volumes in the ranges from 10.1 ml to 75 ml and group 3 represented large infarcts of volume greater than 75 ml. Mean NSE levels in the three groups was compared and it was found that the largest infarct volumes have the highest mean NSE levels [Table 2 and Figure 1]. On comparing, the mean NSE levels in the 3 groups by ANOVA, it was found that there was a statistically significant difference between the 3 groups ($P < 0.001$).

Correlation between NSE concentration and stroke severity at presentation

A strong negative correlation was found between GCS at presentation and concentration of NSE on day 1 ($r = -0.806$, $P < 0.001$) [Figure 2]. Patients with GCS <7 had a higher mean

Table 1: Baseline clinical characteristics, vascular risk factors, neuroimaging findings, and neurological outcome measures of the study population

Variables	Values (%)
Age (mean±SD) years	61.29±12.57
Sex	
Males	47 (62.7)
Females	28 (37.3)
Glasgow coma scale (mean±SD)	7.88±3.28
Hypertension	46 (61.3)
Diabetes mellitus	34 (45.3)
Smoking	42 (56)
Dyslipidemia	23 (30.67)
Blood sugar (mean±SD) mg/dl	192.36±96.32
Infarct volume (measured by CT scan) (mean±SD) ml	53.88±42.92
Serum neuron specific enolase (mean±SD) ng/ml	64.36±49.71
Modified Rankin scale (mean±SD)	3.96±1.50

SD=Standard deviation, CT=Computed tomography

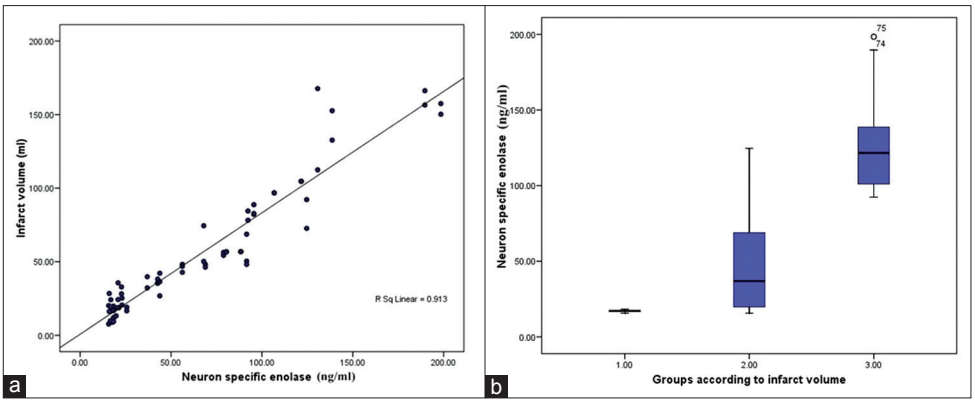

Figure 1: (a) Scatter plot showing correlation between NSE and infarct volume, (b) boxplot showing NSE levels in different infarct volume groups

Table 2: Mean NSE levels according to infarct volume groups

Variable	Group 1 0-10 ml *N*=6	Group 2 10.1-75 ml *N*=50	Group 3 >75 ml *N*=19	*P* value
NSE values (mean±SD) ng/ml	17.04±0.89	44.71±29.07	131.02±36.76	<0.001

NSE=Neuron specific enolase, SD=Standard deviation

NSE levels (109.74 ± 47.20) as compared to patients with GCS was ≥ 7 (37.33 ± 25.86 ng/l). On comparing mean NSE levels in patients with GCS < 7 and GCS ≥ 7 a statistically significant difference was found ($P < 0.001$, $t = -7.48$) [Figure 2].

Correlation between concentration of NSE and functional neurological outcome

Functional neurological outcome was assessed using the mRS at day 30. There was a positive correlation between NSE levels at day 1 and early neurological outcome assessed by mRS at day 30 ($r = 0.744$, $P < 0.001$) [Figure 3]. *mRS* <3 was taken as better outcome and mRS ≥ 3 was considered as worse outcome, concentration of NSE on day 1 was compared between the better outcome and worse outcome group. It was found that the mean concentration of NSE in patients with mRS ≥ 3 was significantly higher as compared to patients with mRS < 3 ($P < 0.001$, $t = 8.49$) [Table 3 and Figure 3].

Multiple linear regression analysis was carried out considering the mRS (functional neurological outcome at day 30) as dependent variable and patient's age, GCS at presentation, concentration of NSE on day 1 and blood sugar as independent variables. It was found that age ($\beta = 0.196$, $P = 0.003$), GCS at presentation ($\beta = -0.628$, $P < 0.001$) and NSE on day 1 ($\beta = 0.291$, $P = 0.011$) were independently associated with outcome measure (mRS).

Discussion

NSE is a dimeric isoenzyme of the glycolytic enzyme enolase with a molecular weight of approximately as 80,000 Da. NSE is found mainly in the neurons and cells of the neuroendocrine system.[2] As NSE is mainly found in neurons its level in the serum or CSF can often be elevated in neurological diseases. González-García *et al*., had very recently observed that serum levels of NSE were significantly higher in patients of acute stroke as compared to controls.[12]

Our study was carried out on 75 patients of acute ischemic stroke, with the aim to determine the correlation between NSE levels on day 1 with infarct volume on day3, stroke severity at presentation and functional neurological outcome on day 30.

We found a positive correlation between NSE levels on day 1 and infarct volume on day 3 ($r = 0.955$, $P < 0.001$). Brea *et al*., studied 224 patients with ischemic stroke and found that NSE serum concentrations at 72 h correlated with infarct volumes determined between the 4th and 7th days (Spearman coefficient 0.456, $P = 0.002$).[1] Wunderlich *et al*., observed that infarct volume determined by CT scan or MRI correlated significantly with NSE at 12 h.[7] Oh *et al*., studied 81 patients with anterior circulation infarction and found a significant correlation between initial serum NSE levels and infarct volume determined by T2 weighted MRI scan ($r = 0.81$; $P < 0.01$).[8] Wu *et al*., also observed that peak NSE levels correlated positively with the infarct volume ($r = 0.81$; $P < 0.01$).[9] Missler *et al*., also reported that Serum levels of NSE correlated with infarct volume ($r = 0.37$, $P < 0.05$).[10] Therefore, this finding of our study was consistent with previous studies.

In our study, stroke severity was assessed using the GCS at presentation, on correlating serum NSE levels with GCS at presentation it was found that there was a strong negative correlation between GCS at presentation and concentration of NSE ($r = -0.806$, $P < 0.001$), it was also observed that patients with lower GCS (<7) had significantly higher NSE levels as compared to patients with higher GCS (≥ 7). Negative correlation between NSE and GCS can be explained by the fact that patients with lower GCS had higher NSE levels and vice versa. These findings are consistent with the study of Brea *et al*., who reported that peak concentration of NSE in serum correlated with stroke severity at admission assessed by the National Institute of Health Stroke Scale (NIHSS) ($r = 0.319$, $P = 0.0001$).[1] Jauch *et al*., also reported that higher 24-h peak concentrations of NSE were associated with higher NIHSS baseline scores ($r = 0.117$, $P = 0.032$).[2] Wunderlich *et al*., observed that NSE levels highly correlated with severity of neurological deficit assessed by NIHSS.[7] According to Oh *et al*., also the initial serum NSE concentrations correlated with NIHSS score at admission ($r = 0.42$, $P = 0.002$).[8] Thus,

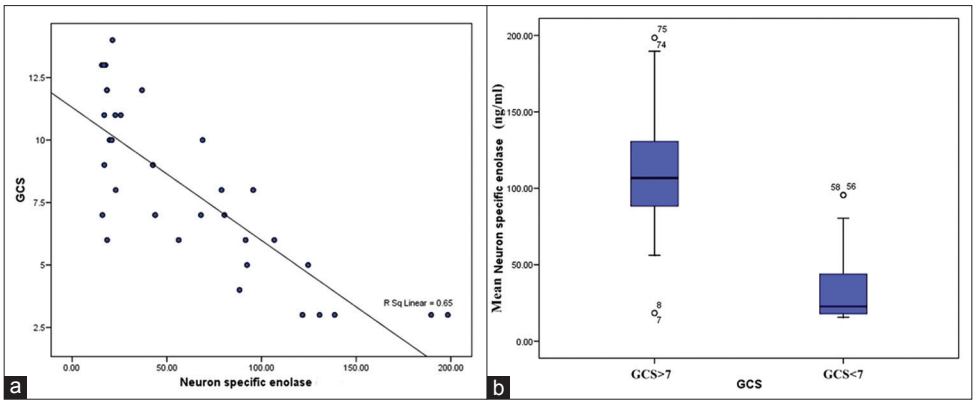

Figure 2: (a) Scatter plot showing correlation between NSE and GCS, (b) boxplot showing mean NSE levels according to GCS

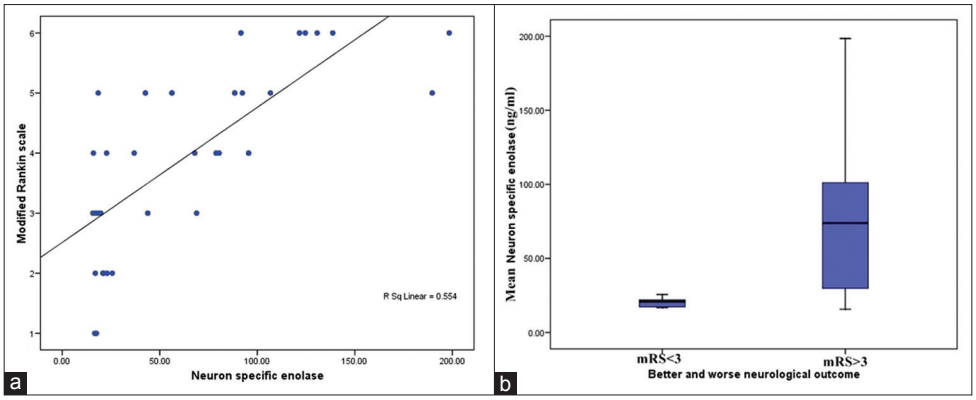

Figure 3: (a) Scatter plot showing correlation between NSE and functional neurological outcome (mRS), (b) boxplot showing mean NSE levels according to better and worse neurological outcome

Table 3: Mean NSE levels in better and worse outcome groups

Variable	mRS<3 *n*=15	mRS ≥ 3 *n*=60	*P* value
NSE (mean±SD) ng/mL	20.32±3.14	75.37±49.81	<0.001

NSE=Neuron specific enolase, mRS=Modified Rankin scale

stroke severity at admission in all these previous studies was assessed using the NIHSS; our study differed slightly from these previous studies as GCS was used to assess stroke severity at admission. The NIHSS is frequently used to quantify stroke severity, but recently some studies have also used GCS to assess stroke severity and outcome.[15] In study, conducted by González-García *et al*., significant correlation was not found between NSE levels and stroke severity on admission, they attributed this difference in results to the fact that timing of blood withdrawal was different in various studies.[12]

In our study, functional neurological outcome was assessed at day 30 using the mRS, it was observed that NSE levels on day 1 correlated significantly with mRS at day 30 ($r = 0.744$, $P < 0.001$), mean NSE concentration in patients with mRS ≥ 3 (worse outcome) was significantly higher as compared to patients with mRS < 3 (better outcome) ($P < 0.001$). It was also found that on multiple linear regression, NSE levels were independently associated with functional neurological outcome (mRS) ($\beta = 0.291$, $P = 0.011$). Similar results were reported recently by González-García *et al*., they assessed functional neurological outcome by NIHSS and found a significant correlation between NSE levels and NIHSS on day 60 ($r = 0.461$; $P = 0.001$), these authors also reported that on multivariate regression analysis, there was an independent association between NSE levels and neurological outcome measure.[12] Brea *et al*., in there study assessed functional neurological outcome using mRS at 3 months, they also reported that patients with poor functional outcome (mRS >2) had significantly greater serum concentration of NSE ($P < 0.0001$) in cases of ischemic stroke, on multivariate analysis NSE at 72 h was independently associated with poor outcome in this study also.[1] Wunderlich *et al*., found that serum NSE levels from 12 h onwards correlated with mRS at 3 months, with maximum correlation obtained for NSE at 96 h ($r = 0.443$, $P < 0.001$).[7] Oh *et al*., predicted short term prognosis using NIHSS score at day 7 and found a significant correlation between initial NSE levels and NIHSS score on day 7 ($r = 0.44$, $P < 0.001$).[8] Similar results were obtained by Wu *et al*., they assessed functional neurological outcome using the Activities of Daily Living scale and found a significant correlation between NSE levels and outcome measure at 1 month ($r = -0.37$; $P < 0.05$), 3 months ($r = -0.45$; $P < 0.01$), and 6 months ($r = -0.65$; $P < 0.001$).[9] Missler *et al*., on the other hand did not find a significant relationship between NSE levels and functional neurological outcome.[10] Thus, our study found results consistent with most of the recent studies.

The major limitation of our study was that NSE levels were estimated only once due to financial constraints, although studies have reported dynamic changes in NSE levels during the first few days after stroke.[1] Therefore, serial measurement of NSE would have provided a better picture of its correlation with different stroke characteristics. Another limitation of our study was that sample size was not very large, multicenter studies with a large number of subjects is required to overcome this limitation. Another shortcoming of the study was the use of GCS to measure stroke severity at admission as NIHSS is more commonly used for the same purpose. Finally, outcome was assessed at 30 days only; the study would have yielded more results if the outcome was reassessed at 3 months and 6 months also.

Thus, to conclude that serum levels of NSE in first few days of ischemic stroke can serve as a useful marker to predict stroke severity and early functional outcome. However, larger studies with serial estimation of NSE are needed to establish these observations more firmly.

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