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Original Article

The Effect of Initial Serum Neuron-Specific Enolase Level on Clinical Outcome in Acute Carotid Artery Territory Infarction

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The prediction of functional outcome in patients with acute cerebral infarction depends on many factors. Various techniques have been applied to predict severity and outcome after cerebral infarction. Neuron-specific enolase (NSE) is a component of a specific brain enzyme and a useful marker of brain injury. We evaluated the relation between initial serum NSE level and short- and long-term clinical outcome in 59 patients with acute cerebral infarction and in 38 age-matched healthy controls. Serum NSE levels were determined in patients with carotid artery (CA) territory cerebral infarction within 24 hours of onset. Brain MRI was performed four to seven days after stroke. Patients were divided into two groups: large CA territory infarction with a lesion extending cortex (cortex group), and small subcortical CA territory infarction (subcortical group) with a lesion confined to the subcortical white matter. We compared the initial serum NSE levels of the two groups. National Institute of Health Stroke Scale (NIHSS) was determined at admission and seven days after onset and the modified Rankin's scale was used at the 3 months follow-up after onset. Serum NSE levels were significantly elevated in patients with acute cerebral infarction compared with the normal controls (13.88 $\pm$ 5.47 ng/dl vs. 8.15 $\pm$ 1.53 ng/dl, $p<0.05$). The initial (<24 h) serum NSE level was higher in the cortical group than in the subcortical group (16.68 $\pm$ 5.70 ng/dl vs. 10.98 $\pm$ 3.34 ng/dl, $p<0.05$). NIHSS on admission and on the 7th day correlated with the initial serum NSE level ($p<0.05$), as were more severe functional outcomes, as determined 3 months after onset ($p<0.05$). This study shows that initial serum NSE level may be a useful marker for severity in acute ischemic stroke, and that it may be well correlated with short-term and long-term functional outcomes.

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INTRODUCTION

Acute cerebral infarction is one of the most important diseases in old age. Because of its long-standing neurological sequelae and of the functional dysfunction it introduces to daily living, prompt treatment and a prediction of prognosis are important. Clinical symptoms and neuroimaging studies have been used to determine the severity of neurological derangement and to predict the neurological outcome in patients with acute infarction. Recent studies have concerned additional markers that might be used to identify the lesion and to predict the size of the infarction and of the prognosis. Several neurobiochemical markers may be used for the evaluation of neuronal injury. Neuron-specific enolase (NSE) is one of these, and has been the subject of many clinical studies and of experiments in animal settings.[1,2] Previous reports have focused on the release and the kinetics of NSE after acute cerebral infarction in humans[3-14] and animals,[15-20] and in other types of brain damage caused by traumatic brain injury,[21,22] hypoxia,[23] cardiac surgery,[24] and status epilepticus.[25-27]

In this study, we measured the initial serum NSE levels in patients with acute carotid artery (CA) territory infarction, and compared these with the severity of neurological deficits and functional outcomes.

MATERIALS AND METHODS

Patients selection and clinico-radiological evaluations

From a consecutive series of 109 patients with acute cerebral infarction admitted to the Department of Neurology, Yongdong Severance Hospital between April and December 2000. We included patients with acute cerebral infarction admitted within 24 hours of the onset and whose lesion was confined to the CA territory by neurological examination on admission, and which was confirmed as a CA territory cerebral infarction by brain MRI during the admission period (range of MRI performance: 4 - 7 days). We excluded those with, hemorrhagic stroke, a history of malignancy, serious neuro-endocrinopathy, symptoms of a transient ischemic attack, vertebro-basilar artery territory infarction, old and multiple cerebral infarction, which could not be distinguished from the new infarct lesion by diffusion MRI and those admitted later than 24 hours after stroke onset.

Fifty-nine patients (38 men and 21 women; mean age, 64.3 ± 13.1 years) were included. All subjects underwent a standardized neurological examination on admission, 7 days after onset, and 3 months after onset for functional outcome. A neurologist blind to all patient information, including the serum NSE level, performed the examination. Neurological deficit was quantified using the National Institute of Health Stroke Scale (NIHSS)[28] on admission and again seven days after onset. Functional outcome was evaluated using the modified Rankin's scale (mRS)[29] 3 months after onset. Age-matched normal controls with no neurological disease, neuro-endocrinological diseases or cancer were selected as controls.

Patients were divided into two groups according to the extent of infarction. The first group consisted of patients whose infarction extended to the cerebral cortex in the CA territory (the cortical group). The second group consisted of patients with a subcortical CA territory infarction (the subcortical group).

Neurobiochemical examinations

All patients' serum samples were collected via intravenous on admission. Samples were allowed to clot, and after centrifuging (5000 rpm, 10 minutes) were stored at -80℃ within 30 minutes for later analysis. Serum NSE was analyzed by enzyme immunoassay (EIA) based on the sandwich technique, which included a solid-phase monoclonal antibody raised against γ, γ-NSE (Cobas® Core II NSE EIA, Roche, Germany). Hemolytic specimens were discarded because the lysis of platelets influenced results.

The assay utilized a highly specific monoclonal antibody to NSE immobilized on a polystyrene bead, in conjunction with a rabbit polyclonal antibody. During the incubation, the NSE reacted simultaneously with the monoclonal antibody bound to the beads and with the rabbit antibody to form the sandwich. The beads were then washed to remove any unbound rabbit antibody and incubated with a highly purified goat antibody to rabbit immunoglobulin conjugated to horseradish peroxidase. Then the goat antibody-horseradish peroxidase conjugates bound to the rabbit antibody which already bound to the beads through NSE. Following this step, the beads were rewashed to remove any unbound antibody-enzyme conjugate and incubated with an enzyme-substrate/chromogen solution. Observed color intensity was directly proportional to the amount of NSE present. Control subjects included 38 age-matched healthy blood donors (25 males and 13 females, mean age: 65.3 ± 1.53 years). Their blood samples were used to determine reference values for NSE concentration (Plasma NSE concentration: 8.15 ± 1.53 mg/dl).

Statistical analysis

We compared the serum NSE levels of patients with acute CA infarction with those of the normal controls using the independent sample T-test. Differences of serum NSE level between the cortical and subcortical groups were also analyzed using the independent sample T-test. The correlation between the serum NSE concentration and the NIHSS score on admission, on the 7th day and at mRS 3 months after onset were evaluated by using regression analysis with Spearman's rank coefficient. Only P-values < 0.05 were considered significant.

RESULTS

The demographic, clinical and neuroradiological patient data with CA territory infarction are shown in Table 1. As compared with normal controls (25 men and 13 women, mean age: 65.3 ± 11.1 years) patients with infarction were no different in terms of age or sex. Thirty-three patients (56%) had hypertension, 10 (17%) had diabetes mellitus, 12 (20%) had heart disease and 8 (14%) had hyperlipidemia. The mean time interval between the onset of infarction and admission was 10.9 ± 10.7 hours. The mean sampling time was 12.9 ± 10.9 hours after onset. The levels of serum NSE were significantly higher in those with acute CA territory infarction than in the normal controls (13.88 ± 5.47 ng/dl vs. 8.15 ± 1.53 ng/dl, $p<0.05$). On comparing the initial serum NSE levels in the cortical and subcortical groups, NSE levels were found to be significantly higher in the former group (16.68 ± 5.70 ng/dl vs. 10.98 ± 3.34 ng/dl, respectively, $p<0.05$).

In terms of an association between the initial level of serum NSE immediately after admission and short-term clinical outcome, we found that higher initial levels of NSE in serum correlated with the severity of neurological deficit (r=0.589, $p<0.05$) (Fig. 1), and that short-term neurological outcome was significantly correlated with the initial level of serum NSE (r=0.667, $p<0.05$) (Fig. 2). At the 3 months follow-up, the initial serum NSE concentration and mRS 3 months after the onset were positively correlated (r=0.635, $p<0.05$) (Fig. 3).

DISCUSSION

NSE is γ, γ-dimer of protein enolase (2- phspho-D-glyceride hydrolase), and a soluble enzyme in the glycolytic pathway with a total molecular weight of approximately 80,000 Da. It represents 1.5% of whole cell soluble brain proteins, and is found predominantly in neurons and neuroendocrine cells.[1] After acute CNS insults, such as

Table 1. Demographic, Clinical, Neuroradiological Data and Serum NSE Concentration in 59 Patients with CA Territory Infarction

	Patients group (%)
Age (year)	64.3 ± 13.1
Sex (M/F)	38 / 21 (64/36)
Risk factor	
Hypertension	33 (56)
Diabetes mellitus	10 (17)
Smoking	23 (39)
Heart disease	12 (20)
Hyperlipidemia	8 (14)
Interval between onset & admission (hours)	10.9 ± 10.7
NIHSS scores at admission	7.9 ± 4.5
NIHSS scores at 7 days after onset	7.0 ± 4.9
mRS scores at 3 months after onset	2.9 ± 1.3
Type of CA territory infarction	
Cortical group	30 (51)
Subcortical group	29 (49)
> 1.5 cm lesion size	14
< 1.5 cm lesion size	15
NSE sampling time (hours)	12.9 ± 10.9
plasma NSE level at admission (ng/dl)	13.88 ± 5.47†

( ): % of total number.
CA, Carotid Artery; NIHSS, National Institute of Health Stroke Scale; mRS, modified Rankin's scale; NSE, neuron-specific enolase.
†Significant difference between patients and normal controls (independent sample T-test, $p<0.05$).

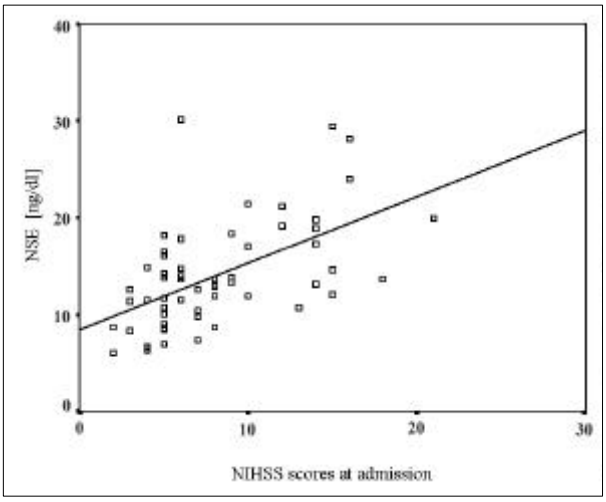

Fig. 1. Scatter plot of serum NSE concentration against NIHSS scores at admission in acute cerebral infarction patients. (Spearman rank correlation coefficient r=0.589, $p<0.05$).

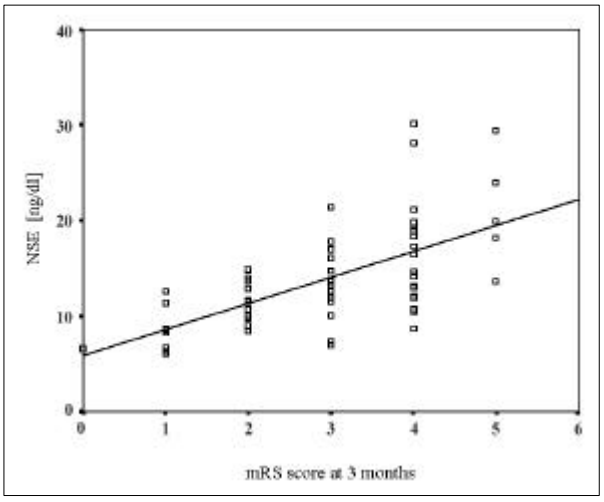

Fig. 3. Scatter plot of serum NSE concentration against modified Rankin's scale scores 3 months after onset. (Spearman rank correlation coefficient r=0.635, $p<0.05$).

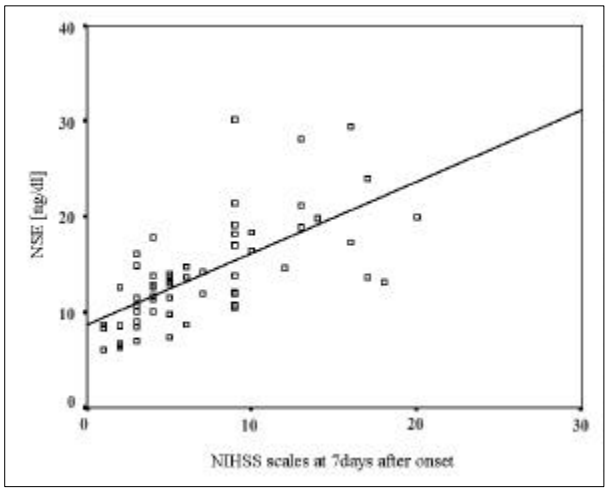

Fig. 2. Scatter plot of serum NSE concentration against NIHSS scores on the 7th day after the onset. (Spearman rank correlation coefficient r=0.667, $p<0.05$).

cerebral infarction, hypoxia, trauma, or seizure, the brain-blood barrier is altered and astroglial disintegration causes NSE to leak into the CSF and serum. Increased NSE into serum and CSF implies neuronal injury. We evaluated the serum NSE level rather than the CSF NSE level because the CSF study would have been invasive and difficult for older patients.

Although the temporal patterns of NSE levels in serum vary widely, according to previous reports, peak levels of NSE in serum are achieved within the first 96 hours after cerebral infarction, and in some cases peak serum NSE levels were found to occur as late as 6 days after infacrtion.[3-5,9,12-14] Patients with acute CA territory infarction in our present study showed increased serum NSE levels within 24 hours of onset versus the normal controls. This result suggests that the analysis of serum NSE during the acute phase of stroke is valuable for evaluating the neurological deficit of cerebral infarction patients.

Some reports have suggested that serum NSE levels predict lesion volume in patients with acute cerebral infarction, and the NSE levels between 48 and 96 hrs after onset were found to be well correlated with infarct size.[4] A previous report showed that patients with total anterior circulation stroke had higher initial NSE serum levels than patients with partial anterior circulation stroke or lacunar infarction.[30] In our study, the initial serum NSE level was significantly higher in patients in the cortical group than in the subcortical group. This suggests that although the exact lesion size was not measured precisely in our present study, that the initial NSE level may be correlated with lesion size.

It is still debatable whether NSE is good biochemical marker of functional outcome in patients with acute cerebral infarction. Several previous studies have failed to demonstrate a significance between NSE and functional outcome after infarc-

tion.[6,9] Some of these evaluated prognosis using the Glasgow Outcome Scale, which has only 5 grades and cannot differentiate patient's detailed outcome. However, a recent study showed that the ratio of peak NSE to carnosinase in 124 patients with ischemic or hemorrhagic stroke is significant correlated with outcome after ischemic stroke, as defined by the Barthel index and the Rankin Scale.[3] Another study showed a positive association between functional outcome (mRS at discharge) and serum NSE concentration after stroke onset in 28 patients with acute cerebral infarction, though no detail was given of mean hospital stay.[31,32] The present study showed also good correlations between the initial serum NSE level with short- and long-term functional outcome. And the results suggest that the patient's prognosis is worse if initial serum NSE level is higher and serum NSE may be used as an indicator of outcome in cerebral infarction patients.

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