Prediction of Early Clinical Severity and Extent of Neuronal Damage in Anterior-Circulation Infarction Using the Initial Serum Neuron-Specific Enolase Level

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Context: Prompt and precise measurement of neuronal damage in acute cerebral infarction is important to determine the prognosis of functional outcome. A feasible biochemical marker such as the neuron-specific enolase (NSE) level has been used to detect various diseases involving the central nervous system.

Objective: To determine whether the initial serum NSE level is a useful marker for predicting the severity of clinical neurological deficits and the extent of neuronal damage in acute anterior-circulation infarction.

Design: Case-control study with biochemical-clinico-radiological correlation.

Setting: Tertiary care center.

Participants: Eighty-one patients and 77 age- and sex-matched control subjects.

Main Outcome Measures: Patients with anterior-circulation infarction underwent intravenous serum NSE sampling within 24 hours after symptom onset. Recent infarction was confirmed by T2-weighted and diffusion-weighted magnetic resonance imaging of the brain about 1 week after the onset of stroke. Volumetric analysis of infarction was also performed. The National Institutes of Health Stroke Scale score was measured on admission to the hospital and 1 week after symptom onset.

Results: The patients’ initial serum NSE levels were statistically significantly higher than the controls ($P < .05$). The initial serum NSE level highly correlated with the volume of infarction seen on T2-weighted magnetic resonance imaging of the brain ($r = 0.62$, $P < .001$) and with the National Institutes of Health Stroke Scale score obtained on hospital admission ($r = 0.42$, $P = .002$) and on the seventh day after the onset of stroke ($r = 0.44$, $P < .001$).

Conclusion: The initial serum NSE level is a reliable predictor for the extent of neuronal damage and the severity of clinical neurological deficits in acute anterior-circulation infarction.

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STROKE IS THE SECOND leading cause of death in South Korea, and most survivors contend with the aftermath of serious functional disability in their daily living. It is important to promptly determine and predict the extent of neuronal damage before treatment of acute cerebral infarction. The assessment of the extent of neuronal damage depends on the type of neuroimaging performed (eg, magnetic resonance imaging [MRI]). The lesion volume by neuroimaging correlates with the clinical neurological deficits, especially in patients with anterior-circulation infarction as shown in previous studies. However, there are some limitations to an MRI evaluation of a patient who has an acute cerebral infarction—the need for the patient to cooperate during the neuroimaging and the inability of a patient with unstable vital signs to cooperate.

Several neurobiochemical markers can evaluate neuronal injury. The serum neuron-specific enolase (NSE) level is one of these markers and is found in the cytoplasm of neurons and cells with neuroendocrine differentiation. Previous reports focused on the release and kinetics of NSE after acute cerebral infarction, traumatic brain injury, hypoxic brain damage, and status epilepticus. However, there are few reports about whether the NSE level in the acute phase correlates with neuronal damage and clinical neurological deficits in anterior-circulation infarction. We investigated whether the initial NSE level within 24 hours after symptom onset is the valuable predictive marker of neuronal damage or clinical function status in patients with acute anterior-circulation infarction.
METHODS

PATIENTS AND CONTROL SUBJECTS

Of 264 patients with acute cerebral infarction admitted to the Department of Neurology, Yongdong Severance Hospital, Seoul, South Korea, between April 1, 2000, and December 31, 2001, 241 patients had MRI studies of the brain to confirm acute cerebral infarction. In this study we included patients who were admitted within 24 hours after symptom onset and whose lesions were only confined to the territory of the internal carotid artery as seen on T2-weighted MRIs of the brain according to previously reported vascular topography. Excluded were patients who were admitted longer than 24 hours after symptom onset, patients with hemorrhagic stroke as seen on computed tomography (CT) of the brain on admission, with evidence of neuroendocrine tumor such as small-cell lung cancer, transient ischemic attack, and vertebrobasilar artery territory infarction. We also excluded the patients with evidence of old cerebrovascular disorders as seen on diffusion-weighted MRI of the brain because we could not be assured as to when the old lesion developed and whether it influenced the serum NSE level. Therefore, 86 patients were included in this study. Additionally, we exclude 5 patients who died within 1 week of symptom onset because of unexplained causes and also because we could not evaluate the seventh day neurological deficits in such patients. Therefore, a total 81 patients were included in this study. We received informed consent from all of the patients enrolled in this study. We selected age- and sex-matched control subjects (n=77) with no evidence of organic brain disease such as tension headache and vestibular neuropathy as seen on MRIs to determine reference values for the concentration of the serum NSE.

CLINICAL ASSESSMENT OF NEUROLOGICAL DEFICITS AND NEUROIMAGING STUDY IN PATIENTS WITH ACUTE CEREBRAL INFARCTION

All subjects underwent standardized neurological examination by 2 experienced neurologists (J.-G.L. and S.-J.N.) on hospital admission and on the seventh day while in the stroke unit. The neurological deficits were quantified using the National Institutes of Health Stroke Scale (NIHSS) score. On admission, hemineurological deficits were measured independently by 2 experienced neurologists (J.-G.L. and S.-J.N.) on hospital admission and on the seventh day while in the stroke unit. The scores were used to calculate the NIHSS score. The NIHSS score was calculated using the extent of lesion on T2-weighted MRI in accord with the Oxfordshire Community Stroke Project classification, that is, total anterior-circulation infarct group, partial anterior-circulation infarct group, or lacunar infarct group. The NIHSS score was calculated using the extent of lesion on T2-weighted MRI in accord with the Oxfordshire Community Stroke Project classification, that is, total anterior-circulation infarct group, partial anterior-circulation infarct group, or lacunar infarct group.

The NIHSS score was calculated using the extent of lesion on T2-weighted MRI in accord with the Oxfordshire Community Stroke Project classification, that is, total anterior-circulation infarct group, partial anterior-circulation infarct group, or lacunar infarct group. All patients had magnetic resonance angiography at the same time. The mechanism of stroke was evaluated according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria. All patients had CT or MRI of the brain, 12-lead electrocardiography, posteroanterior view of the chest, complete blood cell count, and simultaneous multichannel autoanalysis. Digital subtraction cerebral angiography, transesophageal echocardiogram, and 24-hour electrocardiographic monitoring were performed in selected patients.

SERUM NSE COLLECTION AND QUANTIFICATION

All patient samples of NSE were collected via intravenously on admission. Samples were centrifuged for 30 minutes (3000 rpm for 10 minutes) and stored at −80°C for later analysis. The serum NSE sample was analyzed using an enzyme immunoasay based on the sandwich technique including the solid-phase monoclonal antibody raised against γ-γ-NSE (Cobas Core II; Roche Diagnostics, Basel, Switzerland). Hemolytic specimens were discarded because lysis of erythrocytes and platelets influenced the serum NSE level. The assay used the highly specific monoclonal antibody to immobilize NSE on a polystyrene bead in conjunction with the polyclonal antibody of rabbit. During the incubation, the serum NSE reacted simultaneously with the monoclonal antibody bound to the beads and with the rabbit antibody to form the sandwich. The beads were washed to remove any unbound rabbit antibody and subsequently incubated with the highly purified goat antibody to the rabbit immunoglobulin conjugated to horseradish peroxidase. Then the goat antibody–horseradish-peroxidase conjugate was bound to the rabbit antibody already bound to the beads through the NSE. Following this step, the beads were again washed to remove any unbound antibody–enzyme conjugate and were incubated with an enzyme–substrate-chromogen solution. Color intensity was directly proportional to the amount of NSE present in the samples and standard.

VOLUMETRIC ASSESSMENT OF LESION SIZE

The processing of all axial T2-weighted magnetic resonance data was performed using a commercially available computer workstation (Scion Image Beta 4.02; Scion Corp, Frederick, Md). Volumes were measured on the image of maximum contrast between lesions and normal brain regions. Two investigators (S.-H.O. and J.-H.P.) blinded to the clinical symptoms, independently, manually measured the lesion volume; the values were averaged. Interrater correlation was calculated (r=0.97, P<.01). There was a 3-mm-interslice gap, and the volumes of the regions of interest were computed by multiplying the measured area per slice by the section thickness.

STATISTICAL ANALYSIS

Independent t test was used to determine whether there were statistically significant differences in the serum NSE level between patients with acute cerebral infarction and healthy controls using a commercially available statistical software program (SPSS Version 11.0; SPSS Inc, Chicago, Ill). The Spearman product-moment correlation was used to determine the association between the lesion volume on T2-weighted MRI and the serum NSE level on admission in the patient group. Similar calculations were made to compare the significance of the relationship between the NIHSS score and the initial serum NSE level. Data are given as mean (SD) unless otherwise indicated.

RESULTS

In 81 patients (42 males [52%] and 39 females [48%]), the mean age of patients was 66.7 (13.8) years (age range, 48-87 years). In 77 controls (40 males [52%] and 37 females [48%]) the mean age was 63.8 (11.1) years (age range, 52-78 years). No statistically significant differences were noted in age and sex between the patients and controls (P>.05). On evaluation of risk factors of ischemic stroke, 44 patients (54%) had hypertension, 31 (38%) had diabetes mellitus, 39 (48%) had smoked tobacco, 19 (23%) had heart disease, and 9 (11%) had hyperlipidemia.

By T2-weighted axial MRI of the brain, 72 patients (89%) had a lesion of the middle cerebral artery territory infarction. Three patients (4%) had a lesion of the
anterior cerebral artery territory. Six patients (7%) had a border zone infarction, whose lesion was located between the anterior cerebral arterial and the middle cerebral arterial territories. Eighteen patients (22%) were in the total anterior-circulation infarct group, 32 patients (40%) were in the partial anterior-circulation infarct group, and 31 patients (38%) were in the lacunar infarct group. On evaluation using the TOAST classification, 28 patients (35%) had large-artery disease, 13 patients (24%) had cardioembolism, 20 patients (25%) had small-vessel disease, and 20 patients (25%) had undetermined mechanisms (Table). Sixty-eight patients (84%) were treated with heparin sodium, 9 patients (11%) with oral aspirin, and 4 patients (5%) had conservative management without anticoagulant therapy or an antiplatelet agent. None of these patients were treated with thrombolytic agents.

Calculated median lesion volume of patients by T2-weighted MRI was 16.4 mL (range, 1.5-400.4 mL). The level of serum NSE was higher significantly in the patient group (13.0 [5.4] ng/dL) than in the control group (6.3 [1.6] ng/dL) ($P < .05$) (Figure 1). Twenty-two (71%) of 31 patients in the lacunar infarct group, 25 (78%) of 32 patients in the partial anterior-circulation infarct group, and 15 (83%) of 18 patients in the total anterior-circulation infarct group had an elevated serum NSE level above the 98th percentile value of the serum NSE level (8.8 ng/dL) in the control group.

The initial serum NSE levels correlated positively with the volume of infarction ($r = 0.62$, $P < .001$) (Figure 2). The initial serum NSE level also correlated with the NIHSS Scale score on admission ($r = 0.42$, $P = .002$) (Figure 3) and on the seventh day after the symptom onset ($r = 0.44$, $P < .001$) (Figure 4).

**COMMENT**

Neuron-specific enolase (NSE) is the $\gamma\gamma$-dimer of the protein enolase (2-phopho-D-glyceride hydrolase), and soluble enzyme of the glycolytic pathway with a total molecular weight of approximately 80000 daltons. It represents 1.5% of cell-soluble brain proteins and is found predominantly in neurons and neuroendocrine cells. After acute central nervous system insults, such as cerebral infarction, hypoxia, trauma, and seizure, the blood-brain barrier is altered and astroglial disintegration sub-
consistently makes the NSE leak into cerebrospinal fluid and serum. Many previous studies demonstrated an increase in the serum NSE level after acute focal ischemia in humans. In the results of a 1995 study, Creutzfeldt-Jakob disease also increased the NSE level.

There are several previous reports that the infarction size measured on CT scan of the brain correlated with the peak serum NSE level. Our study used MRI of the brain for better measurement of the volume of infarction, and the results also showed that the serum NSE level was increased after acute infarction and well correlated with the lesion volume. However, it is still controversial that whether the elevation of the serum NSE level in patients with acute cerebral infarction is correlated with the clinical disability in the acute or chronic phase. Some reports showed a positive correlation between the serum NSE level and the neurological outcome, whereas others failed to demonstrate the significant correlation. Our results suggested that the initial serum NSE level had some value for predicting clinical severity in acute anterior-circulating infarction.

Wunderlich et al. reported that the concentration of protein S100B, another biochemical marker, seemed to have a higher predictive value than the serum NSE level. However, the elevation of the protein S100B concentration is noted on the second to the fourth day after symptom onset, and its prediction may be delayed in predicting the neuronal damage of the brain and the early clinical outcome in the acute phase of cerebral infarction. Because of time constraint when acute stroke is evaluated, the concentration of protein S100B has some limitation in determining the intensity of treatment in acute cerebral infarction. The serum NSE release in humans peaks earlier on the first through the third day after symptom onset than protein S100B. Therefore, the serum NSE levels can provide earlier information than the S100B protein concentration of the neuronal damage to the treatment of acute cerebral infarction.

Although we could not predict the long-term clinical outcome in patients with anterior-circulation infarction, many studies showed the significant correlation between the clinical outcome and the lesion volume by CT or T2-weighted MRF of anterior-circulation infarction. Recently, a new diagnostic neuroimaging technique, diffusion-weighted imaging with or without perfusion-weighted MRI, in acute ischemic stroke provided the close relationship between the lesion volume and the clinical outcome.

Our study showed that the final volume of infarction measured by MRI had a much closer relationship between the initial serum NSE level and the NIHSS score on admission and on the seventh day after symptom onset. It suggests that the early serum NSE level change is a reliable predictor of the neuronal damage in patients with acute anterior-circulation infarction. By these results, the serum NSE level may be used as one of the simple tests that can be repeated for patients with acute cerebral infarction, especially for those patients with unstable vital signs. This result will provide a good indicator for the more intense neuroprotective treatment in patients with severe neuronal damage in acute anterior-circulation infarction, which may influence the long-term clinical outcome.

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