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SERUM NEURON-SPECIFIC ENOLASE AS A MARKER OF BRAIN ISCHEMIA-REPERFUSION INJURY IN PATIENTS UNDERGOING CAROTID ENDARTERECTOMY

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SUMMARY – In patients with atherosclerotic stenosis of the extracranial segment of internal carotid artery, surgical intervention is an effective method to prevent cerebral ischemic stroke. However, this surgical procedure may cause vascular brain damage. The aim of the study was to investigate consequential brain ischemia-reperfusion injury by measuring the cerebral specific marker, neuron-specific enolase (NSE), in serum of patients having undergone internal carotid endarterectomy (CEA). The study involved 25 patients that underwent CEA due to internal carotid artery stenosis. Blood samples were obtained from each patient on three occasions: within 24 h prior to surgery, 12 h after surgery, and 48 h after surgery. Serum NSE levels were measured by a commercially available enzyme-linked immunosorbent assay. The study showed that serum NSE level was statistically significantly increased 48 h after CEA as compared with the level 12 h after surgery and the level before surgery ($p<0.05$). Difference in serum NSE between the level before surgery and 12 h after CEA was not statistically significant ($p>0.05$). Data from our study showed CEA to affect serum NSE in patients with significant internal carotid artery stenosis. Thus, serum NSE may be used as a biochemical marker of brain ischemia-reperfusion injury following CEA.

Key words: *Carotid endarterectomy; Ischemic stroke; Neuron-specific enolase*

Introduction

Stroke including ischemic stroke is one of the major public health problems worldwide, particularly in industrialized countries. Strokes are a frequent cause of acute hospitalization, severe morbidity, and the third cause of overall mortality[1].

Surgical treatment of atherosclerotic stenosis of the extracranial segment of internal carotid artery is an effective method of preventing cerebral ischemic stroke[2]. However, it should be noted that the procedure is burdened with the consequences of neurological complications during the perioperative period. Microembolisms, macroembolisms, and increased or decreased blood flow to the brain as a result of internal carotid artery endarterectomy (CEA) may lead to ischemic and/or hyperperfusion brain damage[3-5].

Neuron-specific enolase (NSE) is a dimeric isoenzyme of the glycolytic enzyme enolase. NSE has a molecular weight of approximately 80 kDa and is found mainly in the neurons and cells of the neuroendocrine system. It participates in slow axoplasmic transport. NSE is not normally secreted, but when axons are damaged, NSE is upregulated to maintain homeostasis. Therefore, NSE is the only marker that directly assesses functional damage to neurons. As NSE is mainly found in neurons, its level in serum or cerebrospinal fluid (CSF) may be elevated in neurological diseases. According to Jauch *et al*., biochemical markers of acute

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neuronal injury including NSE may play a role in the diagnosis and management of cerebral ischemia. The authors observed increased 24-h peak levels of NSE that were associated with higher than normal baseline scores set forth by the National Institutes of Health Stroke Scale. Moreover, serum NSE may have a prognostic value in traumatic brain injury. Unfavorable outcomes and mortality of patients were significantly associated with increased serum NSE levels. It is also concluded that NSE has a moderate discriminatory ability to predict mortality and neurological outcome in patients with traumatic brain injury[6,7].

Therefore, NSE may also be a biochemical marker of brain ischemia-reperfusion injury in patients undergoing CEA. The aim of the study was to investigate serum NSE levels and its changes over time in these patients.

Patients and Methods

The study involved patients hospitalized in the Department of Vascular Surgery and Angiology, Medical University of Lublin, Lublin, Poland, undergoing CEA due to internal carotid artery stenosis. Patients were qualified for surgical treatment according to the guidelines of the European Society of Vascular Surgery based on Doppler studies performed using a Toshiba Aplio 500 ultrasound[8]. Patients with high grade stenotic carotid arteries were identified and measured by using the guidelines set by the North American Symptomatic Carotid Endarterectomy Trial (NASCET)[9].

The study group consisted of 25 participants (15 male and 10 female) aged from 54 to 88 years, average 69 years. Patients with occlusion of the internal carotid artery were not qualified for surgery and research. The degree of internal carotid artery stenosis ranged from 60% to 90%. The average clamping time of internal carotid artery during CEA was 8.5 minutes. Conventional CEA was performed under local anesthesia. Shunt was not used. No complications associated with CEA were observed. The mean velocity of blood flow in the internal carotid artery was 208.4 cm/s (blood flow 1) before surgery and 89.5 cm/s (blood flow 2) after surgery. Past medical histories of study subjects included previous ischemic stroke (n=8), transient ischemic attack (n=7), and asymptomatic internal carotid artery stenosis (n=10). The study criteria disqualified patients with known organic brain damage in the course of various diseases of the nervous system (e.g., multiple sclerosis, Alzheimer's disease).

Blood samples were obtained from each patient from the antecubital vein on three occasions: within 24 h prior to surgery (NSE 1), 12 h after surgery (NSE 2), and 48 h after surgery (NSE 3). Serum samples were collected into plastic tubes, centrifuged rapidly, and stored at -80 °C until analysis. Serum NSE levels were measured by a commercially available enzyme-linked immunosorbent assay (Enzyme-Linked Immunosorbent Assay Kit for Enolase, Neuron Specific (NSE), Cloud Clone Corp./USCN, Houston, TX, USA).

Statistical analysis was performed using STATISTICA version 10 software (StatSoft, Inc., Poland). Distribution of the collected data was evaluated using the Shapiro-Wilk's test. Furthermore, data on the NSE levels were analyzed using ANOVA with *post hoc* Tukey HSD test. For the dichotomous system, Student's t-test and Mann-Whitney U test (depending on the distribution obtained) were used to examine between-group differences. Correlation analysis was performed using the Spearman rank correlation. The NSE values were expressed in ng/mL. The values of $p<0.05$ were considered significant.

The study was approved by the Ethics Committee of Medical University in Lublin (KE-0254/218/2014).

Results

The ANOVA test showed that sampling time significantly affected serum NSE levels ($p<0.05$). Serum NSE levels and comparative analysis are presented in Table 1.

The *post hoc* Tukey HSD test revealed that serum NSE level was statistically significantly increased 48 h after CEA as compared with the levels measured 12 h after surgery and prior to surgery ($p<0.05$). However, difference in serum NSE levels prior to surgery and 12 h after CEA was not statistically significant ($p>0.05$) (Table 2).

There was no statistically significant difference in serum NSE levels between symptomatic and asymptomatic patients ($p>0.05$). There was no statistically significant correlation between serum NSE levels and clamping time either ($p>0.05$).

There was no statistically significant correlation of serum NSE levels with blood flow velocity in internal

Table 1. Serum neuron-specific enolase (NSE) levels and comparative analysis

	N	NSE level (ng/mL)					p
		Mean	SD	Median	Min	Max	
NSE 1	25	1.34	0.38	1.29	0.43	2.14	<0.001*
NSE 2	25	1.59	0.43	1.53	0.98	2.52	
NSE 3	25	2.00	0.95	1.82	0.31	4.09	

ANOVA test; *statistically significant

Table 2. Comparative analysis of neuron-specific enolase (NSE) levels in patient sera

Difference	Significance
NSE 1 – NSE 2	p=0.2764
NSE 2 – NSE 3	p=0.0338*
NSE 1 – NSE 3	p=0.0005*

Post hoc Tukey HSD test; *statistically significant

Table 3. Correlation of serum neuron-specific enolase (NSE) levels with blood flow velocity in internal carotid artery

Correlation	r	Significance
NSE 1 & blood flow 1	-0.11	p=0.6069
NSE 2 & blood flow 1	-0.15	p=0.4646
NSE 3 & blood flow 1	-0.12	p=0.5624
NSE 1 & blood flow 2	0.11	p=0.5941
NSE 2 & blood flow 2	0.16	p=0.4422
NSE 3 & blood flow 2	0.28	p=0.1693

carotid artery before CEA and after surgery (p>0.05) (Table 3).

However, change in the blood flow calculated as: [(blood flow rate after surgery – blood flow rate prior to surgery)/blood flow rate prior to surgery]*100 and change in the NSE2 and NSE3 levels indicated statistically significant correlations (r=0.45, p=0.0239; and r=0.41, p=0.0394, respectively). The correlation between NSE2/NSE3 levels and blood flow velocity in internal carotid artery is illustrated in Figure 1.

Discussion

Our study revealed that serum NSE level was statistically significantly increased 48 h after CEA as compared with the levels 12 h after surgery and prior to surgery. Dragas *et al.*[10] observed that changes in serum NSE occurred after CEA. The authors concluded that serum NSE could be used as a potential marker of brain injury after surgery. Capoccia *et al.*[11] investigated

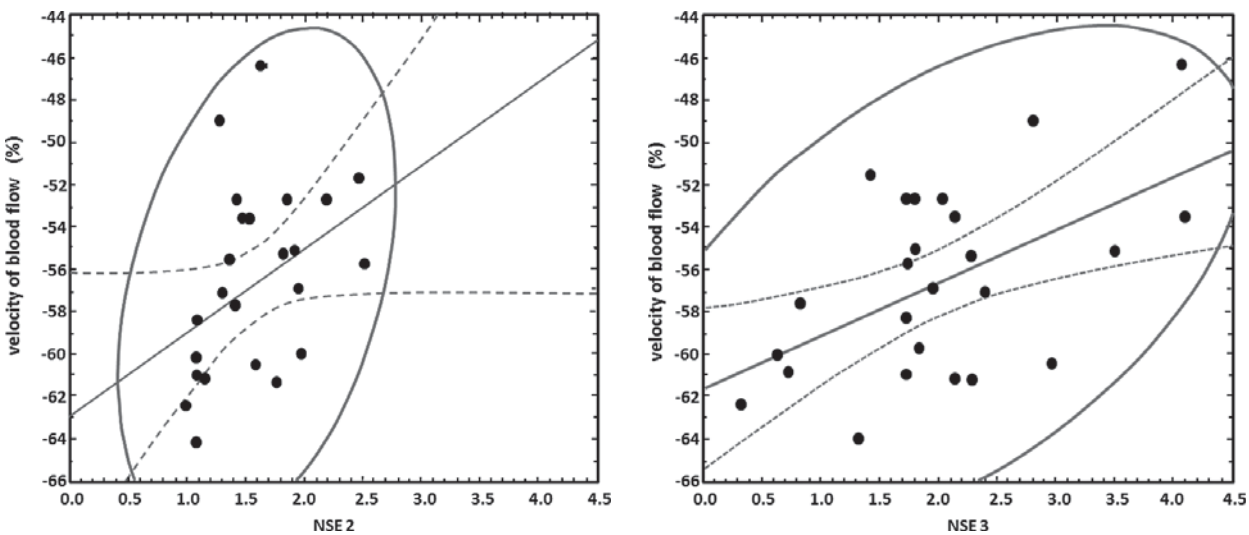

Fig. 1. Correlation between neuron-specific enolase (NSE) NSE 2/NSE 3 levels and blood flow velocity in internal carotid artery.

the relationship between serum levels of two biochemical markers of cerebral injury, S100 protein and NSE, using postoperative diffusion-weighted magnetic resonance imaging, and neuropsychometric testing in patients undergoing CEA or carotid artery stenting (CAS). The authors observed that the aforementioned biochemical markers were significantly increased at 24 h in CAS patients as compared with CEA patients. According to the authors, the increased S100 and NSE levels following CAS may be caused by perioperative microembolization rather than hypoperfusion. Brightwell *et al.*[12] measured serum S-100b and NSE levels in patients undergoing CEA and CAS, and found associated hemodynamic and embolic events detected using transcranial Doppler (TCD). TCD findings showed that the mechanisms of these increased biochemical markers may have differed, and thus may have been caused by microembolization and cerebral hypoperfusion, respectively. According to the authors, cerebral hypoperfusion is responsible for the increase of the above mentioned brain damage markers in cases related to CEA.

Serum levels of NSE were measured intraoperatively, prior to and after carotid clamping. There was no difference in subtle cerebral damage between patients randomized to receive a shunt or not[13]. Gao *et al.*[14] studied NSE levels in serum of patients before, during, and for 2 days after CEA performed under general anesthesia and incorporating the use of a Javid shunt. Serum NSE level increased during shunting, while the CSF NSE level did not change.

Different results were obtained in studies conducted by Rasmussen *et al.*[15]. The authors measured serum levels of NSE in patients before CEA and postoperatively at 12, 24, 36 and 48 h. Compared with abdominal aortic surgery patients, the preoperative serum level of NSE was significantly higher in carotid artery surgery patients. Postoperatively, serum NSE level decreased significantly after uncomplicated CEA, and the level was then similar to that in the aortic surgery patients. The authors concluded that subtle brain damage after carotid artery surgery could not be detected by measuring blood levels of NSE.

The mechanism of serum NSE elevation observed in our study is unknown, but we support the hypothesis that increased blood-brain barrier permeability caused by hypoxia during carotid cross-clamping may influence NSE leaking from the brain into the blood. Cerebral damage during carotid clamping may be the result of microembolism and/or hypoperfusion. Elevated serum NSE levels may reflect ischemia-induced enzyme loss. An increase in serum NSE level may also be caused by hyperperfusion brain damage during CEA. In many patients, clamping of the internal carotid artery during CEA results in transient decrease in cerebral blood flow in the ipsilateral cerebral hemisphere[16]. If this decrease in the hemispheric cerebral blood flow is significant enough to impair autoregulation, then consequently ipsilateral cerebral hyperperfusion can occur after internal carotid artery declamping. Development of cerebral hyperperfusion after CEA is associated with preoperative hemodynamic impairment and intraoperative cerebral ischemia[17]. It has been suggested that acute ischemia and reperfusion by clamping and declamping of the internal carotid artery may produce oxygen-derived free radicals resulting in impairment of cerebrovascular autoregulation, postischemic hyperperfusion, or/and brain edema[18,19].

Brouns *et al.*[20] investigated CSF levels of different neuromarkers including NSE in acute ischemic stroke patients and their relation to initial stroke severity, stroke location, and long-term stroke outcome. They concluded that biomarkers displayed relevant differences in cellular and subcellular origins, which were reflected in their relation to stroke characteristics.

In conclusion, data from our study showed that CEA affected serum NSE in patients with significant internal carotid artery stenosis. Thus, serum NSE may be used as a biochemical marker of brain ischemia-reperfusion injury after CEA.

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Sažetak

SERUMSKA RAZINA ZA NEURON SPECIFIČNE ENOLAZE KAO BILJEG ISHEMIJSKO-REPERFUZIJSKOG OŠTEĆENJA U BOLESNIKA PODVRGNUTIH KAROTIDNOJ ENDARTEREKTOMIJI

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Kirurška intervencija je učinkovita metoda sprječavanja ishemijskog moždanog udara u bolesnika s aterosklerotskom stenozom ekstrakranijskog dijela unutarnje karotidne arterije. Međutim, ovaj kirurški zahvat može uzrokovati oštećenje moždanog krvožilja. Cilj ovoga istraživanja bio je ispitati posljedično ishemijsko-reperfuzijsko oštećenje mozga mjerenjem za neuron specifične enolaze (*neuron-specific enolase*, NSE) kao specifičnog moždanog biljega u serumu bolesnika podvrgnutih endarterektomiji unutarnje karotide (*internal carotid endarterectomy*, CEA). Istraživanje je obuhvatilo 25 bolesnika podvrgnutih CEA zbog stenoze unutarnje karotidne arterije. Uzorci krvi uzeti su u svakog bolesnika tri puta: unutar 24 h prije operacije, 12 h nakon operacije i 48 h nakon operacije. Serumske razine NSE mjerene su komercijalnim testom ELISA. Rezultati su pokazali da je serumska razina NSE bila statistički značajno povišena 48 h nakon CEA u usporedbi s razinom izmjerenom 12 h nakon operacije, kao i u usporedbi s razinom prije operacije ($p<0,05$). Razlika u serumskoj razini NSE prije operacije i 12 h nakon CEA nije bila statistički značajna ($p>0,05$). Podaci dobiveni u ovom istraživanju pokazuju da CEA utječe na razinu NSE u serumu kod bolesnika sa značajnom stenozom unutarnje karotidne arterije. Stoga bi serumska razina NSE mogla poslužiti kao biokemijski biljeg ishemijsko-reperfuzijskog oštećenja mozga nakon CEA.

Ključne riječi: *Endarterektomija karotide; Ishemijski moždani udar; Enolaza specifična za neuron*