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Serum Level of Neuron-Specific Enolase in Patients with Past Ischemic Stroke

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Abstract.

The article presents the results of the examination of 128 patients with past ischemic stroke (on the 4th -5th months after acute cerebrovascular disease).

Enzyme immunoassay for the determination of NSE concentration in blood serum of patients included into the study showed that the median of the distribution of enzyme was 14.3 (5.7; 57.8) ng/ml ($p<0.05$) compared to 3.8 (3.2; 7.5) in patients of the control group.On the basis of the comparison of serum levels of NSE with the value of total NIHSS, MMSE and MoCA score more significant increase in this parameter was detected in patients with profound neurologic deficit and MCI. The analysis of serum NSE is an informative diagnostic criterion for the evaluation of neuronal damage in the brain leading to the inclusion of detection of NSE in clinical practice to optimize diagnostic procedures in patients with past ischemic stroke.

Problem statement and analysis of the recent research

The prevalence and mortality of cerebral stroke and its complications indicate that it is one of the most important medical and social problems in Ukraine and worldwide [1].

Clinical findings and the results of conventional methods of examination may not always reflect the true severity of the condition, the degree of damage to the central nervous system (CNS) and the prognosis of the development of the disease. It justifies the need to search for new markers of early diagnosis of brain ischemia aiming at pathogenetically justified intervention in the pathological process, restoration of normal nervous system activity and reduction in the incidence of disabling conditions.

Over the past decade, functions of neuron-specific proteins (NSP) as markers of damage to the nervous system have been intensively studied in neuroimmunology. Neuron-specific proteins, neuron-specific enolase (NSE) in particular, perform several functions simultaneously: they are involved in myelin formation and synaptic transmission of the nerve impulse; they catalyze specific metabolism, provide molecular mechanisms of learning and memory [7, 10]. NSE is known to be a glycolytic isoenzyme which is primarily found in cells of neuroectodermal origin (in cerebral neurons and peripheral nervous tissue) [3, 4, 6, 13]. The determination of NSE is used in diagnosing and monitoring the efficacy of therapy. It is also a prognostic factor for a range of diseases such as neuroendocrine tumors, oat cell cancer, neuroblastoma, traumatic brain injury, epilepsy. The determination of NSE is helpful after surgeries performed on patients with cardiac pathology as well as after surgical interventions using cardiopulmonary bypass [3, 6, 14]. The promising direction in modern angioneurology is the use of NSE as a tumor marker in patients with acute cerebrovascular disease [3, 4, 6, 7]. In cerebral infarction, hypoperfusion of brain tissues leads to neuronal damage as a result of which NSE and other substances which are defined as markers of neuronal damage release [7]. Cell markers may also release in subarachnoidal hemorrhage due to ruptured aneurysm [7, 14]. NSE is an important marker in evaluating acute complications as it correlates with the degree of neuronal damage and, therefore, is the prognosis of the diseases. NSE increases and reaches its peak values on the first day of stroke. The primary increase in serum levels of NSE is followed by the secondary one which is usually less significant being a result of the secondary damage to brain tissues due to cerebral edema and increased intracranial pressure [7, 13]. The secondary increase in serum levels of NSE may precede clinical signs indicating the progression of the disease [4, 7, 14].

Thus, the study of the concentration of NSE in blood serum of patients with past ischemic stroke (IS) is of great relevance.

The objective of the research was to determine the concentration of marker of brain damage - neuron-specific enolase - in blood serum of patients with past IS.

Materials and methods

There were examined 128 patients with past IS (on the 4th -5th months after acute cerebrovascular disease) at the age of 40-76 years (the average age was 58.5 ± 10.3) The diagnosis was made on the basis of anamnesis and complaints, neurological examination, CT or brain MRI. When performing CT or MRI signs of ischemic lesions of different localization and size were observed in all patients. The etiological factor for stroke was the coexistence of arterial hypertension and atherosclerotic lesion of the extracranial portion of the carotid arteries or diabetes mellitus.

There were 88 males and 40 females. 93 (68.7%) patients were diagnosed with atherothrombotic stroke and 25 (19.5%) patients were diagnosed with cardioembolic stroke. Stroke

was located in the left hemisphere in 77 (60.1%) patients and in 51 (39.8%) patients it was located in the right hemisphere. The control group included 20 practically healthy persons without any signs of cerebrovascular pathology. The study was carried out at the department of vascular neurology of Ivano-Frankivsk Regional Clinical Hospital and the department of neurology of Ivano-Frankivsk City Clinical Hospital No 1. All patients gave informed consent for participation in the study.

The exlusion criteria were reccurent stroke, sensory aphasia, severe somatic pathology.

The degree of neurologic impairment was evaluated using the National Institute of Health Stroke Scale. The following methods of neuropsychological assessment were used: the Mini-Mental State Examination or Folstein test – MMSE (Folstein et al, 1975), the Clock-Drawing Test (Lezak MD, 1983) and the Montreal Cognitive Assessment – MoCA (Nasreddine et al, 2005).

Neuron-specific enolase served as a marker of neuronal damage. Blood sampling was performed in the morning after an overnight fast. The blood was then centrifuged at 2000 rpm. The determination of NSE was performed in obtained serum samples on the analyzer by the method of a solid-phase competitive enzyme immunoassay using human NSE ELISA kits produced by DAI (USA) according to the manufacturer's instructions. The obtained data were statistically processed using software package STATISTICA 6.0. The Mann-Whitney U test, a non-parametric test that is useful for determining two independent variables, was used to assess a statistically significant difference between groups.

Results and discussion

According to the degree of neurologic impairment assigned by the NIHSS at the moment of hospitalization all patients were divided as follows: 45 (35.1%) persons with mild neurological disorders (6.21±1.71 points); 55 (43%) persons with moderate neurologic impairment (10.58±1.04 points); 28 (21.8%) persons with severe neurologic impairment (15.76±1.95 points).

The results of brain MRI/CT revealed the signs of internal or concomitant hydrocephaly, changes in the cerebral hemispheric white matter (focal changes, leukoaraiosis located periventricularly and/or in the subcortical regions of the brain, the prefrontal cortex) generalized expansion of all components of the cerebrospinal fluid system with subsequent brain atrophy.

When studying cognitive functions according to the MMSE scale the average score was 24.2±0.37 points; in the control group this parameter was 29.1±1.9 points ($p<0.05$). The data on the incidence of cognitive impairments (CI) of different severity are presented in Fig.1.

6.25%
29.60%
25%
39%

■ Normal cognitive function
■ Cognitive decline
■ Mild post-sroke dementia
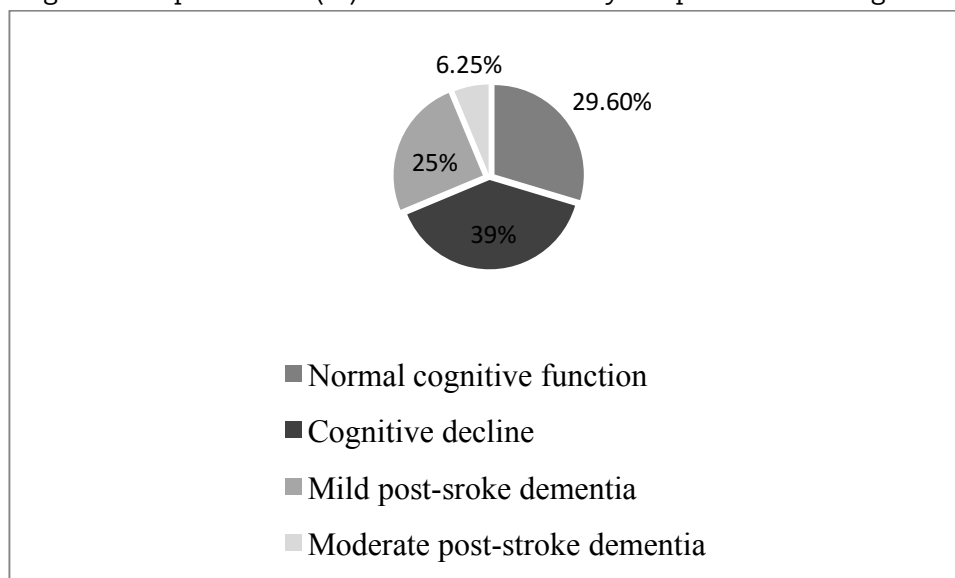■ Moderate post-stroke dementia

Fig.1. Incidence of cognitive impairments of different severity according to the MMSE scale

Enzyme immunoassay for the determination of NSE concentration in blood serum of patients included into the study showed that the median of the distribution of enzyme was 14.3 (5.7; 57.8) ng/ml ($p<0.05$) compared to 3.8 (3.2; 7.5) in patients of the control group (Table 1).

Table 1

Serum level of NSE in blood of patients with past ischemic stroke in different degrees of neurological disorders

Neurologic impairment according to the NIHSS, points	NSE, ng/ml	NSE, ng/ml	
		Control group (n= 20)	Patients with past IS (n= 128)
Mild (n=45)	10.25 (5.7; 19.2)*		
Moderate (n=55)	13.9 (6.8; 35)*	3.8 (3.2; 7.5)	14.3 (5.7; 57.8)
Severe (n=28)	16.9 (11.2; 57.8)*		

Notes:

The data are presented as a median (the 25th and 75th percentiles);

*Statistically significant difference compared to the control group ($p<0.05$)

Therefore, patients with past IS were divided into two groups: Group I included patients with serum NSE levels less than 14 ng/ml (n=68) and Group II included patients with serum NSE levels greater than or equal to 14 ng/ml (n=60). When comparing cognitive functions lower neuropsychological test scores were observed in patients with serum NSE levels greater than 14 ng/ml compared to the control group and patients with less serum levels of NSE (Table 2).

Table 2

Comparison of parameters of cognitive functions in patients with past IS and those of the control group, (M ± m)

Parameters	Group I	Group II	Control group
MMSE	26.36±0.2*	24.15±0.19*^	29.1±1.1
MoCA	24.04±0.17*	21.6±0.21*^	28.05±0.24
Clock-Drawing Test	8.9±0.4	8.1±0.5*	9.2±0.8

Notes:

The data are presented as a median (the 25th and 75th percentiles);

*Statistically significant difference compared to the control group ($p<0.05$);

^Statistically significant difference compared to Group I ($p<0.05$)

There were strong correlations between serum level of NSE and the MMSE score (R=-0.78; $p<0.05$) and between NSE and the MoCA score (R =-0.71; $p<0.05$). The obtained data may indicate the possibility of using the determination of NSE concentration in blood serum for the validation of diagnosis of an increase in manifestations of post-stroke CI and the development of post-stroke dementia.

On the basis of the comparison of serum levels of NSE with the value of total NIHSS score more significant increase in this parameter was detected in patients with profound neurologic deficit (Table 1). Thus, in group of patients with severe neurologic impairment the median of the

indicator of NSE was 16.9 (11.2; 57.8, p<0.05) that exceeded the value of this indicator in the control group by 25% being probably caused by a greater extent of focal changes (Fig.2).
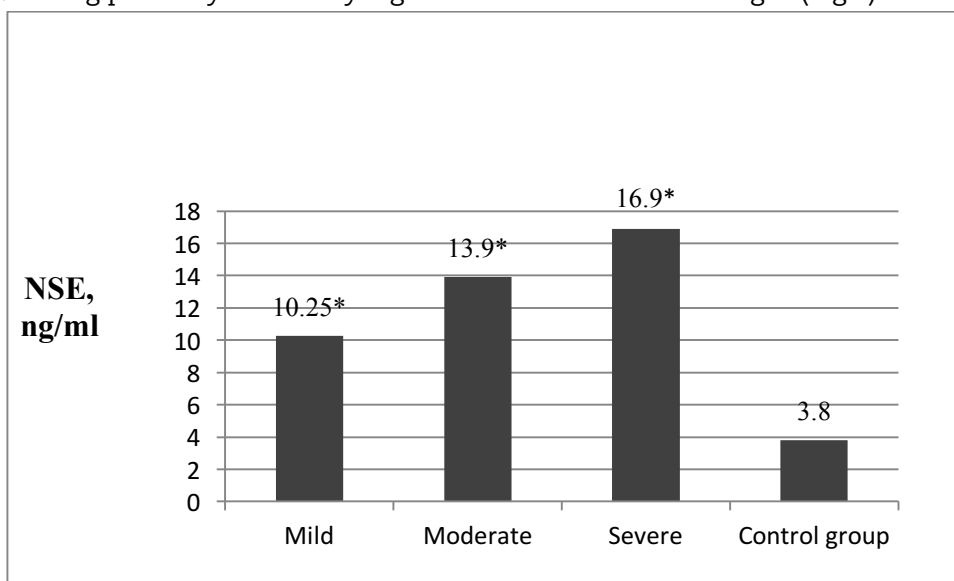

Fig.2. NSE level according to the degree of neurologic impairment assigned by the NIHSS
Note:
*Statistically significant difference compared to the control group (p<0.05)

There was a direct correlation between serum NSE and the severity of neurologic impairment (R=0.71; p<0.05) indicating the direct dependence of the degree of post-ischemic brain damage and neurological disorders. NSE directly reflects the depth and intensity of structural and functional biomembrane abnormalities in the central nervous system, the severity of pathomorphological changes in neurons and the degree of blood-brain barrier permeability [11, 12].

The analysis of the concentration of NSE in blood serum revealed its increase in patients with co-existent somatic diseases, type II diabetes mellitus (type II DM) and ischemic heart disease (IHD) in particular (p<0.05) (Table 2). This dependence was absent in patients with chronic lower limb venous insufficiency (CLLVI).

Table 3

Concentration of NSE in blood serum depending on the presence somatic diseases

Parameter	Somatic diseases			Control group
	IHD	Type II DM	CLLVI	
NSE level, ng/ml	11.5 (4.8;20.1)*	16.4 (4.9; 24.5)*	11.2 (4.3; 19.4)	4.7 (3.25; 7.55)

Notes:
The data are presented as a median (the 25th and 75th percentiles);
*Statistically significant difference compared to the control group (p<0.05)

Thus, the determination of serum NSE is an informative diagnostic criterion for the evaluation of neuronal damage in the brain.

Conclusions

1. The increase in serum level of NSE in patients with past ischemic stroke depending on the severity of cognitive and neurologic impairment being of diagnostic value was established.

2. The correlations between serum level of NSE and the severity of neurologic impairment (R=0.71; p<0.05) and between the MMSE score (R=-0.78; p<0.05) and the MoCA score (R =-0.71; p<0.05) were detected.

3. The deterioration in cognitive function and high serum levels of NSE are predictors of unfavorable prognosis.

4. The results indicate the informative value of NSE level in patients with past ischemic stroke in determining the degree of severity leading to the inclusion of detection of NSE in clinical practice to optimize diagnostic procedures in in this cohort of patients.

Prospects for further research

Considering the prevalence and variety of clinical manifestations of this pathology the promising direction is the study of the influence of changes in the concentration of markers of post-ischemic damage and adhesion molecules on dynamics of neurologic state and cognitive impairments in patients with past ischemic stroke undergoing complex neuroprotective therapy as well as the development of appropriate prognostic criteria to improve the effectiveness of therapeutic measures in this cohort of patients.

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