Neuron-specific enolase level in patients with metabolic syndrome and its value forecasting acute stroke

Oral Ospanov1,2,3, Kadisha Ospanova4, and Irina Kadyrova5,6

1. Department of Laparoscopic & Bariatric Surgery, Medical University of Astana, Astana, Kazakhstan
2. Society of Bariatric and Metabolic Surgeons of Kazakhstan, Kazakhstan
3. University Medical Center, Kazakhstan
4. Karaganda Stroke Prevention Centre, Karaganda, Kazakhstan
5. Department of Physiology, Karaganda State Medical University, Karaganda, Kazakhstan
6. Laboratory of Collective Use, Karaganda State Medical University, Karaganda, Kazakhstan


ABSTRACT

Background
Patients with metabolic syndrome are at a greater risk of experiencing a cerebrovascular event. Several studies show that patients with metabolic syndrome have asymptomatic ischemic brain injury. In this case, there is a need for rapid determination of asymptomatic brain lesions and prediction of acute stroke.

Aims
The aim of the study was to determine the neuron-specific enolase (NSE) serum level in patients with metabolic syndrome and the value of this level for forecasting acute stroke.

Methods
The study used the following information to determine metabolic syndrome: waist circumference, total cholesterol, triglycerides, high-density lipoprotein cholesterol, blood pressure, and blood glucose. Doppler sonography mapping of the brachiocephalic trunk was held to determine the percentage of the carotid artery stenosis. To determine asymptomatic ischemic brain injury, the NSE serum marker was measured. Statistical processing of the measurements was performed using the H test and the Mann–Whitney test. The possible link between MS and NSE were determined by logistic regression analysis. Mathematical modeling was performed using logistic regression.

Results
There are statistically significant differences in NSE concentrations in groups with metabolic syndrome and ischemic stroke patients. This assertion is confirmed by logistic regression analysis, which revealed the existence of a relationship between metabolic syndrome and increased concentration of NSE.

Conclusion
Patients with metabolic syndrome have an increased concentration of NSE. This indicates the presence of asymptomatic ischemic neuronal damage. A prognostic model for determining the probability that patients with metabolic syndrome will have an acute stroke was developed.

Key Words
Metabolic syndrome, ischemic stroke, neuron specific enolase

What this study adds:
1. What is known about this subject?
The MS in interconnection with NSE serum concentration before stroke manifestation has not been investigated yet as a forecasting factor on the territory of Kazakhstan and worldwide.
2. What new information is offered in this study?
Investigation revealed increased concentration of NSE in MS patients’ serum and significant role of mutual determination of NSE and carotid artery stenosis in stroke forecasting.

3. What are the implications for research, policy, or practice?
Use of a proposed prognostic technique will help to detect asymptomatic brain damage in patients with high risk of occurrence of stroke during screening surveys.

**Background**

The incidence of ischemic stroke is one of the most important health and social problems throughout the world due to the high level of morbidity and mortality. Along with the issues concerning the treatment and rehabilitation of people who have suffered a stroke, there is the relevant aspect of its prediction. It is known that predicting stroke and assigning the correct treatment allows avoiding a lethal outcome for the patient and reducing the degree of disability. The most simple and accessible means for predicting stroke are prognostic models.

Patients with metabolic syndrome (MS) are a special group at risk of cerebrovascular problems. They are characterized by the following features: high blood pressure, insulin resistance and/or increased levels of blood glucose, abdominal obesity, and dyslipidemia. Often these patients have elevated levels of uric acid, CRP, and altered hormonal profiles. All these factors trigger and maintain the formation of atherosclerosis, a major cause of cardiovascular diseases.

The study described in Diabetes Care shows that brain injury was found in patients with MS asymptomatic ischemic. The authors used neuroimaging techniques (MRI) to detect abnormalities.

Serum markers have been widely adopted in patients with cerebrovascular accidents in mind of the availability of biomaterial and a great diagnostic value. One of the most studied markers in the diagnostic and prognostic aspects is neuron-specific enolase (NSE).

There are three types of enolases, one of which is neuron specific. NSE is an enzyme consisting of two gamma subunits. The biochemical and physiological role of NSE is in the catalysis of the transition of 2-phospho-D-glyceric acid to phosphoenolpyruvate, which is the penultimate reaction of glycolysis. It is found in the cytoplasm and dendrites of neurons and neuroendocrine cells, in the case of brain damage due to increased blood-brain barrier (BBB) permeability and neurodegeneration concentration of NSE in blood serum arise. Elevated NSE usually reflects the severity of the dysfunction of the BBB and possesses a high predictive value for the occurrence of cerebral ischemic stroke. The highest concentration of NSE was observed 4–8 hours after brain damage and was directly proportional to the severity of neuronal damage. There are publications devoted to the study of NSE in newborns experienced intrauterine hypoxia. According to the authors, the increased concentration of NSE in children is characterized by the presence of brain lesions, but there is a temporary absence of clinical manifestations. Relying on these studies, NSE was selected in our research to characterize the processes of degradation of cerebral tissues and dysfunction of the BBB in participants.

However NSE also associated with such pathologies as small cell lung cancer, neuroblastoma, neuroendocrine tumours, melanoma, seminoma, renal cell carcinoma, Merkel cell tumour, carcinoid tumours, dysgerminomas, malignant phaeochromocytoma and immature teratomas. These data added extra requirements for the inclusion and exclusion of patients in the study in order to avoid the presence of patients with benign and malignant tumors.

There was no data that allowed the determination of NSE in MS patients before the manifestation of stroke.

The aim of our study was to determine NSE serum level in MS patients to describe its concentration and prognostic value in the development of acute stroke.

The scientific novelty of the research was to establish a relationship between the presence of MS and the increased concentration of NSE in patients.

**Method**

**Sample frame and sample size calculations**

Initial sample included 384 participants. The final sample however included 157 people formed randomly, because patients with no data of the blood’s biochemical analysis and with the lack of the one of metabolic syndrome’s component were removed. Also those whose blood was subjected to lysis were excluded. The study involved two subsets of patients: 1) patients who did not suffer an acute stroke and 2) patients who experienced an ischemic stroke. All participants in the study were divided into four groups for comparison of independent group criterion of Kruskal-Wallis and Mann-Whitney U-test.
The first group (control) consisted of 38 healthy individuals. The criteria for inclusion in the control group were: age 50–80, blood pressure within normal ranges 120/80 millimeters of mercury, body mass index (BMI) of 18.5–24.9, blood lipidogram (TG <1.7mmol/l; HDL-Ch levels in men >1.03mmol/l, in women >1.29mmol/l; LDL-Ch <2.6mmol/l; total cholesterol 3.5–5.2) and glucose level (3.3–5.5mmol/l) corresponding to the norm.

The second group of patients (with MS) comprised 39 participants. Diagnosis of metabolic syndrome was carried out according to International Diabet Federation (IDF) criteria (2005)\(^{13}\).

The third group was made up of 44 male and female patients who were aged 50–80 and who had suffered an ischemic stroke (IS). The fourth group consisted of 36 patients who had suffered an ischemic stroke and has MS.

IS patients were meant as a clinical syndrome presented by focal and / or general cerebral disorders due to sudden cessation of blood supply to a particular brain region with the following death of brain tissue.\(^{14}\) The procedure for forming the groups is shown in Figure 1.

Patients who had experienced an ischemic stroke were observed the first 12–72 hours after manifestation. Blood sampling was carried out according to the recommendations of the reagent kit to determine the NSE in the first 12 hours.

All participants of the study passed through an annual screening, including the determination of blood cancer markers and clinical and instrumental research methods (colonoscopy, fluorography, x-ray mammography in women),\(^{15}\) which allowed us to include patients without benign and malignant tumors. This undertaken measure excludes the effect of tumor cells on the concentration of blood NSE of the subjects.

Informed consent was obtained for the examination of patients. Personal privacy rights were observed. The sample included patients visiting the City Centre of Primary Health and Stroke Centre of Karaganda.

**Recruitment methods**

Examination included questionnaires, measurement of anthropometric data, and blood tests.

Systolic (SBP) and diastolic (DBP) blood pressure (BP) was measured using a monometer with obligatory compliance for correct registration of blood pressure. Waist circumference measurements were carried out by using a measuring tape, and the results were evaluated in centimeters. Blood samples from the cubital vein for laboratory analysis were performed on patients who had fasted according to standard conditions. Biochemical blood tests were carried out in the laboratory of the Regional Medical Center with the required internal and external control. Blood tests were conducted on a BioSystemA-15 biochemical analyzer using Vital reagents. Determination of NSE was conducted at the Laboratory of Collective Use (LCU) of Karaganda State Medical University. Blood was collected by Vacutainer vacuum systems with gel to separate the serum. After being sampled, the blood was centrifuged and transported to the LCU in an hour for further study. The lysed samples were excluded from the study. NSE ELISA (Fujirebio) sets of reagents were used to determine the marker. Linked immunosorbent assay was performed using the Tecan Evolizer 100. NSE were measured in ng/ml, normal reference according to the Fujirebio ELISA set instruction was 0–12ng/ml.\(^{16}\)

Doppler sonography mapping of the brachiocephalic trunk was performed by an X8 MEDISON SONOACE scanner being used to determine the percentage of carotid artery stenosis (CAS percentage) and following references being used.\(^{17}\)

Blood lipidogram included determination of total cholesterol (TCh), triglycerides (TG), high-density lipoprotein cholesterol (HDL-Ch), and low-density lipoprotein cholesterol (LDL-Ch) by standard methods on a biochemical analyzer. The results were evaluated in mmol/l. Determination of blood glucose (BG) was performed after 12 hours of fasting by collection of capillary blood from the finger. It was measured by express method using a MEITER Optium Xceed glucometer. The results were evaluated in mmol/l. Metabolic syndrome was diagnosed according to the IDF criteria (2005): abdominal obesity (waist circumference in men >94cm; in women >80cm) and any two of the following characteristics: 1) TG ≥1.7mmol/l; 2) HDL-Ch levels in men <1.03 mmol/l, in women <1.29mmol/l or lipid lowering therapy; 3) SBP ≥130mmHg or DBP ≥85mm Hg or antihypertensive therapy; 4) fasting glycaemia ≥5.6mmol/l.\(^{13}\)

**Data collection and analysis**

Statistical processing of the measurements was performed using the SPSS 20 software package (SPSS Inc, Chicago, IL). Verification of distribution normality was carried out using descriptive statistics, quantile charts, and the Kolmogorov–Smirnov test. Not all data in groups had normal distribution.
The presence of MS will be increased in 1.305 times with a significance level of p=0.001.

In group 2 we observe a significant increase in the concentration of NSE in comparison to group 1, the control group. The difference between group 2 (patients with MS) and group 3 (stroke patients) is not significant. This implies that the neuronal damage in MS patients corresponds to neuronal damage in patients with ischemic stroke. This assertion is confirmed by logistic regression analysis, which revealed the existence of a relationship between MS and increased concentration of NSE.

There were no significant differences between groups 3 and 4. This finding suggests that the presence of MS in people who have suffered an acute stroke does not affect the concentration of the marker.

Discussion

The results of our study show that patients with MS may have asymptomatic ischemic damage of cerebral neurons and disintegration of the blood–brain barrier (BBB). Significant output of NSE through the damaged plasmatic membranes of brain cells indicates the intensity of structural and functional disorders of brain neurons. Such damage can be attributed to the presence of mild ischemia. According to Sala et al., the brain damage in patients with MS has a diffuse character and is associated with the degree of progression of MS. The authors demonstrated that the sum of MS risk factors leads to microstructural damage of white and gray matter of the brain and is associated with diffuse changes rather than global macrostructural disorders or processes of demyelination. The diffuse changes discovered in the microstructure of the gray matter of the brain are the result of ischemia. The results of a Japanese study show that MS is associated with three asymptomatic brain lesions: silent brain infarction, periventricular hyperintensity, and subcortical white matter lesions. The authors suggest that the metabolic changes that accompany MS lead to the defeat of arteries in the brain, and eventually to asymptomatic brain damage.

We assume that our results in patients with MS have the following explanation. Components of MS (hypertension, dyslipidemia, insulin resistance, abdominal obesity) lead to a chronic proinflammatory status and constant circulation of cytokines, including TNF-α. This leads to endothelial dysfunction and abnormal BBB permeability. Hypertension and dyslipidemia affect the small arteries of the brain, which leads to the disruption of metabolic processes in
nearby astrocytes and neurons. Arteriosclerosis then leads to chronic hypoxia. In addition, large quantities of cytokine-rich plasma (including TNF-α cytokine) filter through the damaged BBB. TNF-α induces an increased release of glutamate, which leads to excitotoxicity and disruption in the flow of neurons. Besides, processes activated by hypoxia, such as lipid peroxidation, mitochondrial dysfunction, and impairment in energy metabolism, trigger neuronal apoptosis. This leads to the destruction of neurons, and NSE enters the bloodstream. In acute ischemic brain lesions, neurons die by necrosis, but subacute ischemia leads to apoptosis. Apoptotic processes are therefore maintained for four weeks. This explains the constant concentration of NSE in patients with MS (group 2). Astrocytes are more resistant to hypoxia due to the large supply of glycogen, reduced glutathione, the availability of increased synthesis of adenosine, and the anti-inflammatory factor HIF-1alpha. This increases their resistance to ischemia and prevents apoptosis. According to Ruth et al., abdominal obesity, dyslipidemia, and high fat diet leads to glial activation and triggers neuronal apoptosis. The mechanisms of these processes are still incomprehensible. When a stroke or brain trauma occurs, astrocytes start an inflammatory cascade to prevent neuronal death. However, during prolonged dyslipidemia, massive gliosis occurs, astrocyte function is depleted, and prevention of the apoptosis of neurons becomes impossible. Perhaps because of the exhaustion of patients with MS due to prolonged dyslipidemia, astrocytes do not trigger the inflammatory cascade and cannot prevent neuronal apoptosis.

Summarizing data in the literature and the results of our research, we can assume that the risk factors in patients with MS lead to asymptomatic lesions of brain neurons due to mild ischemia. Such damage does not lead to cell necrosis, but initiates and maintains a diffuse neuronal apoptosis. This is the reason for increased NSE concentration. Since apoptosis does not trigger massive inflammatory and destructive reactions, the area of ischemic damage is absent and the depletion of astrocytes is present in response to dyslipidemia. Japanese researchers in their work propose using the link between MS components and asymptomatic brain damage as a diagnostic tool for the prediction and prevention of acute stroke. Relying on the results of our study, we developed a mathematical model for predicting the probability of ischemic stroke occurring in patients with MS.

The event stroke in patients with MS correlated with the following parameters: CAS percentage ($r=0.588$, $n=75$, $p<0.001$), SBP ($r=0.585$, $n=75$, $p<0.001$), DBP ($r=0.472$, $n=75$, $p<0.001$), and BG ($r=0.284$, $n=75$, $p=0.013$). The correlation coefficient with the marker NSE was also significant ($r=0.541$, $n=75$, $p<0.001$). Prognostic model for occurrence of stroke in MS patients developed by the method of logistic regression allows one to determine the probability of a stroke in a patient or to estimate the degree of the influence of factors we investigated on the probability of a stroke. The regression coefficients obtained are summarized in Table 3.

Since the p-value for the regression coefficients for such parameters as SBP, DBP, and BG is greater than 0.05, they are not included in the mathematical model for predicting the probability of ischemic stroke occurring in patients with MS. The prognostic model included two parameters: CAS percentage and the concentration of the NSE marker of brain neuronal damage. Adjusted regression coefficients for CAS percentage and NSE are shown in Table 4.

Thus the probability of ischemic stroke occurring in patients with MS can be determined by two parameters: CAS percentage and NSE. Acting on this finding will allow preventive treatment to be carried out, providing the ability to timely prevent acute ischemic stroke.

The equation for determining the probability of ischemic stroke occurring in patients with MS is:

$$y = \frac{\exp(-7.794+0.130*x_{CAS}+0.250*x_{NSE})}{1+\exp(-7.794+0.130*x_{CAS}+0.250*x_{NSE})};$$

The likelihood coefficient -2 LL is 58,087, which indicates an improvement in the accuracy of the model by 16,741, than when using only the constant -2 LL=74,828. The $R^2$ Nagelkerk criterion equals to 0.709 indicates that the prognostic model using NSE and CAS, accounts for 70% of the variance.

The strength of the study: was found the influence of MS on the NSE concentration without a manifestation of the stroke. For the first time in Kazakhstan, the relationship between asymptomatic brain lesion and metabolic syndrome was studied. The limitations of the study: is the lack of conducting a parallel examination of patients with MS and participants of the control group by neuroimaging methods (CT/ MRI).

Future research could include the parallel detection of neurospecific markers, examination of MRI, and determination of the cognitive functions in patients with MS. It is also necessary to study the effect of
neuroprotective therapy on the brain and concentrations of neurospecific proteins in patients with MS.

Conclusion

Neurospecific markers in patients with MS were determined for the first time. Patients with MS have increased concentration of NSE, which is a marker of neuron damage in the brain. This indicates the presence of asymptomatic ischemic neuronal damage and violation of the integrity of the BBB and implies the need for neuroprotective treatment before the manifestation of acute ischemic attack. In the presence of an acute cerebrovascular event, MS does not affect the concentration of NSE in serum.

A prognostic model for determining the probability of ischemic stroke occurring in patients with MS was developed.

After further research, it would be possible to recommend NSE as a screening method for determining asymptomatic brain damage in MS patients.

References

14. Ministry of Health and Social Development of the Republic of Kazakhstan. Clinical protocols. References. Archive; 2016 from 27 December. Protocol No. 18 https://diseases.medelement.com/disease/%D0%B8%D1%88%D0%B5%D0%BC%D0%BB%D1%87%D0%B5%D1%81%D0%BA%D0%B8%D0%B9-%D0%B8%D0%B0%D1%81%D1%83%D0%BB%D1%8C%D1%82-2016/14928.


ACKNOWLEDGEMENTS
The authors are grateful to the staff of medical organizations of Kazakhstan for essential contribution to the realization of the State Program "Health of Kazakhstan". Furthermore, they are pleased to all branches of primary sections of City Centre of Primary Health and Stroke Centre of Karaganda.
Figure 1: Sample formation

Table 1: Descriptive characteristics of groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N (%)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>57.5(53–65.2)*</td>
<td>57(52–66)*</td>
<td>63.7(10.3)**</td>
<td>65(9.3)**</td>
<td></td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>54(34.4)</td>
<td>31(81.6)</td>
<td>5(12.8)</td>
<td>11(30.6)</td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>103(65.6)</td>
<td>7(18.4)</td>
<td>34(87.2)</td>
<td>25(69.4)</td>
<td></td>
</tr>
<tr>
<td>Waist circumference</td>
<td>–</td>
<td>79(73.8–84.5)*</td>
<td>98.02(14.96)**</td>
<td>82.09(12.3)**</td>
<td>96(90.7–99.7)*</td>
</tr>
<tr>
<td>BG</td>
<td>–</td>
<td>5(4.6–5.2)*</td>
<td>5.6(5.0–5.9)*</td>
<td>5.9(4.8–6.6)*</td>
<td>6.25(5.4–8.9)*</td>
</tr>
<tr>
<td>HDL-Ch</td>
<td>–</td>
<td>1.35(1.25–1.59)*</td>
<td>1(0.88–1.1)*</td>
<td>1.3(0.44)**</td>
<td>1.04(0.9–1.4)*</td>
</tr>
<tr>
<td>SBP</td>
<td>–</td>
<td>120(110–120)*</td>
<td>130(120–140)*</td>
<td>140(120–150)*</td>
<td>160(140–170)*</td>
</tr>
<tr>
<td>DBP</td>
<td>–</td>
<td>80(70–80)*</td>
<td>80(80–90)*</td>
<td>90(80–90)*</td>
<td>90(90–100)*</td>
</tr>
<tr>
<td>TC</td>
<td>–</td>
<td>1.12(0.39)**</td>
<td>1.79(0.94)**</td>
<td>1.38(0.88–1.55)*</td>
<td>1.89(1.3–2.64)*</td>
</tr>
<tr>
<td>CAS percentage</td>
<td>–</td>
<td>0.0(0.0–11.25)*</td>
<td>25.0(20.0–35.0)*</td>
<td>45.0(35.0–50.0)*</td>
<td>45.0(35–50.0)*</td>
</tr>
<tr>
<td>NSE</td>
<td>–</td>
<td>5.39(2.23)**</td>
<td>9.43(5.7)**</td>
<td>15.9(2.7)**</td>
<td>15.12(12.9–17.3)*</td>
</tr>
</tbody>
</table>

* – Characteristic of parameters with a distribution that differs from the norm: median (percentiles 25 and 75)

** – Characteristic of parameters with normal distribution: the mean (SD)

Table 2: Results of comparing NSE markers in four groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Kruskal-Wallis criterion</th>
<th>( \chi^2 )</th>
<th>Degree of freedom</th>
<th>\textit{p}</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSE</td>
<td>88.498</td>
<td>83.861</td>
<td>3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 3: Coefficients of logistic regression for parameters with a significant correlation with the event stroke in patients with MS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n β</th>
<th>n OR</th>
<th>n 95% CI</th>
<th>p</th>
<th>a β</th>
<th>a OR</th>
<th>a 95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS percentage</td>
<td>0.119</td>
<td>1.126</td>
<td>1.068–1.187</td>
<td>p&lt;0.001</td>
<td>0.178</td>
<td>1.195</td>
<td>1.065–1.341</td>
<td>p=0.03</td>
</tr>
<tr>
<td>SBP</td>
<td>0.74</td>
<td>1.077</td>
<td>1.037–1.117</td>
<td>p&lt;0.001</td>
<td>0.66</td>
<td>1.068</td>
<td>0.985–1.157</td>
<td>p=0.111</td>
</tr>
<tr>
<td>DBP</td>
<td>0.111</td>
<td>1.118</td>
<td>1.053–1.187</td>
<td>p&lt;0.001</td>
<td>0.69</td>
<td>1.072</td>
<td>0.934–1.230</td>
<td>p=0.324</td>
</tr>
<tr>
<td>BG</td>
<td>0.219</td>
<td>1.244</td>
<td>0.984–1.573</td>
<td>p=0.68</td>
<td>0.337</td>
<td>1.401</td>
<td>1.006–1.952</td>
<td>p=0.046</td>
</tr>
<tr>
<td>NSE</td>
<td>0.252</td>
<td>1.286</td>
<td>1.133–1.460</td>
<td>p&lt;0.001</td>
<td>0.193</td>
<td>1.213</td>
<td>1.001–1.471</td>
<td>p=0.049</td>
</tr>
</tbody>
</table>

Table 4: Logistic regression coefficients for the parameters CAS percentage and NSE

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n β</th>
<th>n OR</th>
<th>n 95% CI</th>
<th>p</th>
<th>a β</th>
<th>a OR</th>
<th>a 95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS percentage</td>
<td>0.119</td>
<td>1.126</td>
<td>1.068–1.187</td>
<td>p&lt;0.001</td>
<td>0.130</td>
<td>1.139</td>
<td>1.065–1.217</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>NSE</td>
<td>0.252</td>
<td>1.286</td>
<td>1.133–1.460</td>
<td>p&lt;0.001</td>
<td>0.250</td>
<td>1.284</td>
<td>1.106–1.491</td>
<td>p=0.001</td>
</tr>
</tbody>
</table>