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ASSOCIATION BETWEEN SPECIFIC ENOLASE SERUM LEVELS AND OUTCOME ACUTE ISCHEMIC STROKE ONE MONTH AFTER ONSET

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ABSTRACT

Stroke is the second leading cause of death a disability in the world. The disease has a great impact on the social environment and the economic burden for the patient, so it requires great effort for the experts to treat appropriately. Specific markers play a role in the diagnosis, determination of risk factors, and severity of ischemic stroke. One such markeris the Neuron-Specific Enolase (NSE) level, which was thought to reflect the severity of brain damage in stroke patients. The aim of this research was to determine the association between levels of serum specific enolase and outcome acute ischemic stroke one month after onset. This study had a cross-sectional design, consisting of 77 patients with acute ischemic stroke admitted to the Department of Neurology Dr. M. Djamil Hospital Padang, July 2016 until August 2017. Each patient tested for NSE serum and the Modified Rank in Scale (mRS) score was assessed one-month after the onset of ischemic stroke. The Spearman test used to determine the correlation between two variables. P-value of <0.05 was considered statistically significant. There were 42 males (54.54%) and 35 female (45.46%) with a median age 58.21 (16-88) and median levels of NSE 5.94 (2.77-36.75) μg/L. The median mRS score of one-month onset was three (1-6). There was an association among serum NSE levels and outcome of ischemic stroke onset one-month (r = 0.286, p-value = 0.012, R2 = + 8.2%). Based on the study results, NSE levels were associated with damage to the brain parenchyma and could assess the outcome of stroke one-month later.

Key words: Neurons specific enolase, ischemic stroke, outcome

INTRODUCTION

Stroke is a global health problem, since it is the second leading cause of death and the primary cause of disability in the world. The disease has a significant impact on the social environment and economic burden of the patient, so it requires a great effort of the experts to understand the underlying pathogenesis and to seek the best treatment.[1]

Data from the World Health Organization (WHO) in 2015, showed that every year 15 million people worldwide suffer from a stroke. Globally, stroke is the second leading cause of death over the age 60, and the fifth leading cause of death in people aged 15 to 59 years old. In 2010, the incidence of ischemic stroke was higher than the bleeding strokes. The mortality rates of ischemic stroke and bleeding stroke in the low- and middle-income countries were 57% and 84%.[2]

Some ideal biochemical markers that can be used to diagnose, monitor, and to determine the prognosis of a stroke. That marker should meet the following criteria: specific to the brain, detectable in the patient's blood during an acute stroke, rises immediately within hours of an attack, the peak levels reflect the extent of brain damage, and can become a detection instrument for the future prognosis of the disease. The pathophysiology of stroke is a complex process, involving mechanisms of excitotoxicity, damage due to the oxidative processes, ion balance disorders, apoptosis, angiogenesis, neuroprotection, and inflammatory mechanisms. A few numbers of biomarkers have been studied including proteins, peptides, cytokines, chemokines, metabolites, leukocytes, platelets, progenitor cells, microparticles, and others.[3,4]

The Neurons-Specific Enolase (NSE) is one of the biomarkers involved in the stroke pathology, it is found mainly in the cytoplasm and neuroendocrine cells, but it also can be found in other tissues. Increased levels of NSE in the blood are found in some acute abnormalities of the central nervous system, such as cerebral infarction, subarachnoid hemorrhage, head injury, hypoxia, seizures, and cardiac arrest. This condition occurs due to the destruction of blood-brain barrier accompanied by the damage of neuronal cells caused by a leakage of NSE which can be detected in the cerebrospinal fluid, saliva or blood.[3,5]

The neuron-specific enolase is a dimericiso enzyme of the glycolytic enzyme enolase that is found in the cytoplasm of neurons and cells with

differentiation of neuro endocrine. This enzyme is often proposed as a specific neuro biochemical marker for brain damage after an ischemic onset of the human brain. Many studies have been done about the relationship between NSE and acute ischemic stroke severity. Several studies have suggested that the amount of NSE in cerebrospinal fluid correlated with the volume of infarction.[6,7]

An increased of NSE levels are thought to be associated with the infarction volume and the extent of brain tissue damage clinically seen from the severity of stroke.[8] An increase of NSE levels in the cerebrospinal fluid, blood or saliva also can be found in some acute abnormalities of the central nervous system, such as cerebral infarction, head injury, hypoxia, and seizures. Neuron-specific enolase levels are not only increased after nervous system damage but can also increase in neuro endocrine cells cancer such as small cell lung cancer, neuroblastoma, melanoma, and carcinoid tumor.[9]

Increased NSE levels in stroke occur due to neuronal cell damage that can be detected in the cerebrospinal fluid or blood. Neuronal damage and cell membrane disturbance will disrupt of the blood-brain barrier and disintegration of the astroglial cells, so NSE will passively diffuse into the extracellular.[10]

METHODS

This research was an observational study using across-sectional method. Subjects of this study were ischemic stroke patients from July 2016 to August 2017 in the Neurological Ward, M. Djamil Hospital. The criteria inclusion was that the patient had an ischemic stroke with onset less than 48 hours from diagnosis by taking history, neurologic examination, and Gajah Mada score algorithm. Subjects had to sign informed consent paper as an agreement to participate in this study. If patients had neurologic improvement less than 24 hours, they must be excluded. Other exclusion criteria were head trauma, intracranial tumor, epileptic state, CNS infection and Parkinson, sepsis, encephalopathy, thyroid tumor, and lung carcinoma. There were 808 stroke patients, among them 382 patients were diagnosed as ischemic stroke, but only 80 patients could be included as subjects. During the research, three patients were excluded because of no contact. Data was taken such as NSE serum level and Modified Rankin Scale (mRS) score in the first month after onset. All data were analyzed using correlation test.

RESULT AND DISCUSSION

The study was performed on ischemic stroke patients in Neurologic Ward M. Djamil Hospital. Sample, data were taken from July 2016 until May 2017, and NSE serum level was analyzed in June 2017.

Based on sex, this study found that the incidence was higher in males 42 people (54.54%) than females 35 people (45.46%). Characteristics of the subjects and the results of the study can be seen in Table 1. Age range from 77 people in this subject was between 16 to 88 years old. From the analysis of data distribution

Table 1. Subject characteristics and study results

Variable (n= 77)	Median (min-max)
Age	58.21 (16-88)
Glasgow coma scale score at admission	13 (8-15)
Blood sugar levels at admission (mg/dL)	171.18 (89-402)
NSE Levels (ng/mL)	5.94 (2.77-36.75)
NIHSS score at onset < 48 hours	6 (1-22)
mRS score at one-month after the onset	3 (1-6)

Table 2. The variable analysis which influenced NSE level

Variable	N	NSE level (ng/mL)	P
Age			
15-45	11	4.69 (3.08 – 14.38)	0.24
46-88	66	6.49 (2.77 – 36.75)	
Gender			
Male	42	6.71 (3.31-36.75)	0.34
Female	35	5.67 (2.77-24.71)	
Random blood glucose level			
Hyperglycemia (GDS < 200 mg/dL)	20	6.83 (2.77-24.71)	0.91
Normoglycemia (GDS = 200 mg/dL)	57	5.60 (3.06-36.75)	

normality by using the Kolmogorov-Smirnov test, an un even distribution of each variable was obtained, because the data was transformed to be normally distributed. Therefore, the Spearman test was used.

Based on some literature, it was noted that the NSE level was not influenced by age and gender analyzed in this research. In Table 2 it can be seen that there was no correlation between NSE levels with age group and gender when the ischemic stroke happened.

Correlation between NSE serum levels in the acute phase with mRS ischemic stroke score in one-month onset can be seen in Figure 1. Correlation test with Spearman showed r-value =0.286, power of correlation was moderate and p-values = 0.012. There was a correlation between NSE serum levels with acute ischemic stroke outcome by using mRS scores. Correlation between two variables was positive, R^2= + 8.2%.

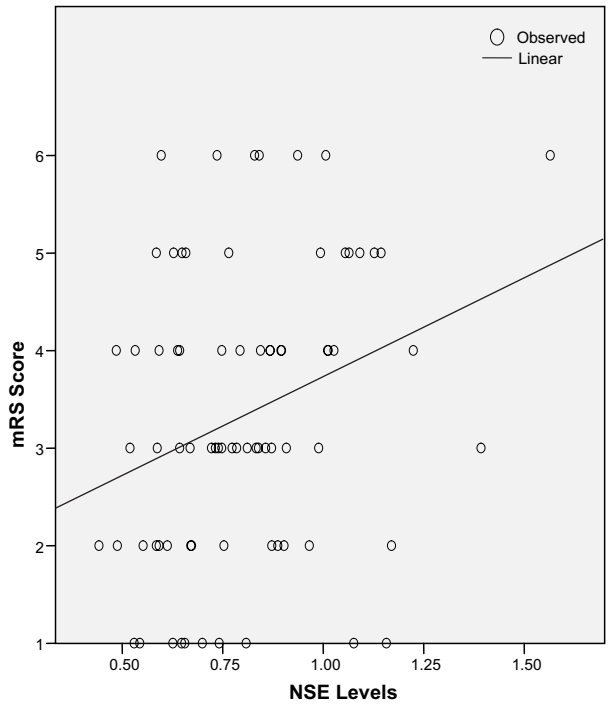

Figure 1. Scatter-plot between NSE serum level on acute phase(onset < 48 hour) with ischemicstroke outcome (one-month after onset) by using mRS; r = 0.286, p-value= 0.012, R^2= +8.2%

In this research, subject age average was 58.21 years old, ranged between 16 years old until 88 years old. This characteristic was almost similar to Pandey *et al*. with age average (59.71 ± 12.6). In contrast, research held by Kaca-oryriska *et al*. showed the age average 71±8, age range between 45 until 77 years old. Altunayoglu *et al*. also showed a sample age average about 66.1± 12.8 years old. Differences among sample age average are caused by the geographic condition, races, and lifestyles.[10]

The number of male subjects in this study was 52 people (54.5%). The incidence of stroke increases with age and occurs more in older males but not at a young age. Comparison of incidence between males and females at age 55-64 years was 1.25, at age 65-74 years was 1.50, at age 75-84 years was 1.07, and at age ≥ 85 years was 0.76.[11] These results were similar with research by Brea *et al*., with 224 subjects studied and 69.6% males.[12] The process of atherosclerosis could occur at various ages but increases in old age. Some risk factors could occur at any age but increased with age. The five major risk factors contributing to the incidence of stroke were as much as 80%, such as hypertension, current smoking status, central obesity, diet, and physical activity.[13] The presence of incidence differences between males and females is also thought to be related to protective effects of estrogen and influence of sex hormones.[14]

The NSE serum level means of the study (onset <48 hours of ischemic stroke) was 7.39 µg/L with median 5.94 µg/L where the lowest level was 2.77 µg/L, and the highest level was 36.75 µg/L. This finding was different from the study conducted by Wu *et al*., which was done on 38 subjects who obtained NSE levels 18.48 ± 16.61 ng/mL. While research conducted by Sri, on 43 subjects showed serum levels of NSE was 11.41 ± 5.07 ng/mL. While NSA serum levels were assessed in a normal population by Casmiro *et al*., where the study assessed serum NSE and cerebrospinal fluid levels in the normal population of 108 subjects showing NSE content was 8.7 ± 3.9 ng/mL (p = 0.06).[15-17] In this study, one of the inclusion criteria was patients coming within 48 hours after onset, so that NSA serum levels were obtained within the first 48 hours after the incidence of the infarction. The NSE examination was not done serially, only once checked when the patient came to the hospital, so the researchers could not determine the peak time of serum NSE levels of patients. This difference might also becaused by the inspection procedure performed. A study conducted by Wunderlich *et al.* found that first serum NSE peak levels were found at the time of onset, followed by a second increase from day two to day four, where the first NSE peak is in 7-18 hours after stroke onset. The normal levels of NSE also varied, because of different determination methods.[18] In this study, the ELISA technique with the Quanticine ELISA for Human Enolase 2/ Neuron-Specific Enolase from R & D Systems was used, while Casmiro research used Immunofluorescent assay monoclonal Antibody)

with TRACE technology.

A study that was conducted by Padalkar in 2014 on 60 acute stroke patients at onset <72 hours and the other 60 as control subjects, with the result of NSE levels in ischemic stroke patients, was increased compared to control subjects (p <0.05), with 87.10% sensitivity, 95% specificity. A study conducted by Omar mentioned that the serum NSE concentration was correlated significantly with the degree of disability due to stroke according to the volume of infarction in acute stroke patients at onset <24 hours. In Indonesia, Noor found a correlation between serum levels of NSE with SSGM score (Spearman correlation = 0.596, p = 0.000).[19-21] While Missler *et al*., Wu *et al*. and Kaca-oryriska *et al*. found that NSE serum levels increased in stroke patients, but were not correlated with severity and stroke outcomes.[15,22,23] Research conducted by Marangos showed that NSE had a high sensitivity and was not influenced by age and gender. This study, after the statistical tests were done, no correlation between NSE levels with age, and blood sugar at the time was found.

The correlation between serum NSE level of acute phase (onset <48 hours) with ischemic stroke outcome function (one-month after onset) in this study showed low correlation strength (r = 0.286) but had a significant correlation between serum levels of NSE and external function acute ischemic stroke using mRS score (p-value = 0.01). A positive relationship between NSE levels with mRS score (R2 = 8.2%) was found. This was in accordance with a study conducted by Hill *et al*., where NSE levels were significantly correlated with NIHSS score at admission (p <0.05) and mRS score (p <0.005). This also corresponded to several other studies.[24] This study results that NSE levels were associated with brain parenchymal damage and could assess the functioning outcome of a stroke after one-month.

In contrast to a study conducted by Wu *et al*., showing that serum NSE was elevated in acute ischemic stroke patients but negatively correlated with functional outcome function at one-month (r = -0.37, p <0.05), month 3 (r = -0.45, p <0.01) and six-month (r = -0.65, p <0.001).[15] Missler *et al*. found that serum NSE increased to fourteen stroke onset and was correlated with infarct volume (r = 0.37, p <0.05) but not significantly correlated with outcome function.[22]

CONCLUSION AND SUGGESTION

There was a correlation between NSE serum levels with ischemic stroke outcome function at one-month of onset. It is recommended that subsequent studies assess the disease complications that affect functional outcomes.

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REFERENCES

1. Thrift AG, Cadhilhac D, Thayabaranathan T, Howard G, Howard V, *et al*. Global stroke statistics. International Journal of stroke, 2013; 9(1): 6-18.
2. Krishnamurthi R, Moran A, Feigin V, Barker CS, Norrving B, *et al*. Stroke prevalence, mortality and disability-adjusted life years in adults aged 20-64 years in 1990-2013: Data from the global burden of disease 2013 study. Neuroepidemiology, 2015; 45(3): 190-202.
3. Whiteley W, Li CW, Sengupta A, Sandercock P. Blood markers for the prognosis of ischemic stroke, a systemic review. Stroke, 2009; 40: e380-e389
4. Rodriguez YM, Sobrino T, Aria S, Vazquez HF, Brea D, *et al*. Early biomarkers of clinical-diffusion mismatch in acute ischemic stroke. Stroke, 2011; 42: 2813-18.
5. Antonietta IM, Bottoni P, Scatena R. Neuron-specific enolase as a biomarker: Biochemical and clinical aspects. Springers Science, 2015; 98-110.
6. Wu YC, Zhao YB, Lu CZ, Qiao J, Tan YJ. Correlation between serum level of neuron-specific enolase and long-term functional outcome after acute cerebral infarction: A prospective study. Hong Kong Med J, 2004; 10(4): 251-4.
7. Stanca DM, Marginean IC, Soritau O, Muresanu DF. Plasmatic markers for early diagnostic and treatment decisions in ischemic stroke. Journal of Medicine and Life 2015; 8:21-25.
8. Stevens H, Jacob C, de-Jager AEG, Cunningham RT, Korf J. Neuron-specific enolase and N-acetyl-aspartate as potential peripheral markers of ischemic stroke. European Journal of Clinical Investigation. 1998; 29: 6-11.
9. Prasad BO, Tiwari S, Usman K. Role of serum neuron-specific enolasein differentiating the side of brain lesion in acute ischemic stroke. International Journal of Biomedical Research, 2015; 6(08): 570-573.
10. Brouns R, De V, De SD, Marien P, De DP. Neurobiochemical markers of brain damage in cerebrospinal fluid of acute ischemic stroke patients. Clinical Chemistry, 2010; 56: 451-58.
11. Altunayoglu V, Gunduz A, Karaca Y, Alioglu Z, Mentese A, *et al*. Diagnostic significance of ischemia-modified albumin, S100b, and neuron-specific enolasein acute ischemic stroke. JAEM, 2014; 13: 112-7.

12. Rosamond W, Flegal K, Friday G, Furie K, Go A, *et al.* Heart disease and stroke statistics-2007 update: A report from the American Heart Association Statistic Committee and Stroke Statistics Subcommittee. Circulation, 2007; 115(5): 69-171.
13. Brea D, Sobrino T, Blanco M, Cristobo I, Rodriguez GR, *et al.* Temporal profile and clinical significance of serum neuron-specific enolase and S 100 in ischemic and hemorrhagic stroke. Clin Chem Lab Med, 2009; 47(12): 1513-18.
14. O'Donnell M, Xavier D, Liu L, Zhang H, Chin S, *et al*. Risk factors for ischemic and intra cerebral hemorrhagic stroke in 22 countries (the INTERSTROKE study): A case-control study. The Lancet, 2010; 376(9735): 112-123.
15. Ritzel R, Capozzi L, Mc Cullough L. Sex, stroke and inflammation; The potential for estrogen-mediated immuno protection in stroke. Hormones and Behavior, 2013; 63(2): 238-53.
16. Wu YC, Zhao YB, Lu CZ, Qiao J, Tan YJ. Correlation between serum level of neuron-specific enolase and long-term functional outcome after acute cerebral infarction: A prospective study. Hong Kong Med J, 2004; 10(4): 251-4.
17. Sri WN, NurdjamanN, NaniK. Korelasi peningkatan kadar neuron spesifik enolase dengan derajat keparahan dan luaran fungsional pasien stroke infark trombotik akut. MKB, 2010; 42(2): 62-8.
18. Casmiro M, Maitan S, De Pasquale, Cova V, Scarpa E, *et al*. Cerebrospinal fluid and serum neuron-specific enolase concentrations in normal population. European Journal of Neurology. 2005; 12: 369-374.
19. Wunderlich TM, Claus W, Michael G. Release of neurobiochemical markers of brain damage are related to the neurovascular status on admission and the site of arterial occlusion in acute ischemic stroke. Journal of the Neurological Sciences, 2004; 227: 49-53.
20. Padalkar R, Sangita P, Sonali B, Rahul G, Dhananjay A. Study of neuron-specific enolase as potential biomarker for assessing the severity and outcome in patients with cerebrovascular accidents. Global Journal of Medical Research, 2014; 14(3): 1-7.
21. Ahmad O, Wardlaw J, Whiteley W. Correlation of levels of neuronal and glial markers with radiological measures of infarct volume in ischemic stroke: A systemic review. Cerebrovasc Dis, 2012; 33(1): 47-54.
22. Komariah N, Setyawati, Usi S. Korelasi neuron-spesific enolase (NSE) serum dengan kejadian perburukan pada pasien stroke iskemik akut. Thesis. 2011.
23. Missler U, Wiesmann M, Friedrich C, Kaps M. S-100 protein and neuron-specific enolase concentrations in blood as indicators of infarction volume and prognosis in acute ischemic stroke. Stroke, 1997; 28(10): 1956–60.
24. Kaca-Orynska M, Tomasiuk R, Friedman A. Neuron-specific enolase and S 100B protein as predictors of outcome in ischemic stroke. Neurologia Neuro chirurgia Polska, 2010; 44(5): 459-63.
25. Hill M, Jackowski G, Bayer N, Lawrence M, Jaeschke R. Biochemical markers in acute ischemic stroke. Canadian Medical Association Journal, 2000; 162(8): 1139-40.