CONTENTS

RESEARCH

Differences of Plasma Interleukin-6 and Tumor Necrosis Factor-A Levels in Healthy People, Rifampicin Resistant and Sensitive Pulmonary Tuberculosis Patients
Wahyu Setiani Wibowo, Jusak Nugraha, Soedarsono ................................................................. 129 - 134

Association between Specific Enolase Serum Levels and Outcome Acute Ischemic Stroke One Month After Onset
Yuri Haiga, Darwin Amir, Yuliarni Syafrita ......................................................................................... 135 - 139

Analysis of Hemoglobin Levels And Leukocyte Count in Neonates with Hyperbilirubinemia
Dewi Suharti, Sulina Yanti Wibawa, Muthmainnah ........................................................................... 140 - 144

Diagnostic Value of Ca-125 in Patients with Epithelial Ovarian Cancer at the Dr. Soetomo General Hospital Surabaya in 2016
Kintan P. R. Kania, Betty A. Tambunan, Willy Sandhika .................................................................... 145 - 149

Analysis of Vitamin D in Patients with Type 2 Diabetes Mellitus
Arfandhy Sanda, Uleng Bahrur, Ruland DN. Pakasi, Andi Makbul Aman .................................................. 150 - 154

Proportion of Rhesus Blood Phenotypes at the Blood Donor Unit in Bandung City
Ivana Dewi, Nadjwa Zamalek Dalimoenthe, Anna Tjandrawati, Nida Suraya ........................................... 155 - 160

Correlation of Total Lymphocyte Count with CD4 Count in HIV/TB Coinfected Patients
Herniaty Rampo, Uleng Bahrur, Mansyur Arif ...................................................................................... 161 - 164

Using Six Sigma to Evaluate Analytical Performance of Hematology Analyzer
Robiul Fuadi ................................................................................................................................. 165 - 169

Correlation of AA Index with Degree of Liver Fibrosis in Chronic Hepatitis B Patients
Rika Andriany, Ibrahim Abdul Samad, Mansyur Arif ............................................................................ 170 - 173

Difference in HbA1c Level between Boronate Affinity and Ion Exchange-High Performance Liquid Chromatography Method in Diabetic Patient
Tuti Asyran, Ellyza Nasrul, Rikarni, Tutty Prihandani ........................................................................... 174 - 179

Diagnostic Value of Neutrophil Lymphocyte Ratio to Differentiate Ischemic and Hemorrhagic Stroke
Martina Rentauli Sihombing, Liong Boy Kurniawan, Darwati Muhadi .................................................... 180 - 183

D-Dimer and Fibrinogen in Patients Underwent Surgery in Malignant and Benign Ovarian Tumor
Ismail Aswin, Herman Hariman, Fauzie Sahil ....................................................................................... 184 - 190
Relationship between Specific Gravity of Cupric Sulfate and Saturation of Blood Droplets During Donor's Hemoglobin Screening
Resna Hermawati, Solichul Hadi
.......................................................................................................................................................... 191 - 193

Vancomycin-Resistant *Staphylococcus aureus* at the Dr. Wahidin Sudirohusodo Hospital Makassar
Fatmawaty Ahmad, Nurhayana Sennang, Benny Rusli
.......................................................................................................................................................... 194 - 198

The Levels of Interleucin-6 (IL-6) and Tumor Necrosis Factor Alpha (TNF-ALFA) in Preeclampsia Patient and Normal Pregnancy
Mawardi, Ratna Akbari Ganie, Sarma N. Lumbanraja
.......................................................................................................................................................... 199 - 201

Analysis of Platelet Volume Mean, Platelet Distribution Width, and Platelet Count in Hemorrhagic and Non-Hemorrhagic Stroke
Gita Medita Sunusi, Darwati Muhadi, Mansyur Arif
.......................................................................................................................................................... 202 - 206

High Fluorescent Lymphocyte Count Examination in Dengue Hemorrhagic Patients with Sysmex Xn-1000 Hematology Analyzer
Budiono Raharjo, Solichul Hadi
.......................................................................................................................................................... 207 - 210

Prevalence and Characteristics of Multidrug-Resistant *Acinetobacter baumannii* Cases at the Dr. Wahidin Sudirohusodo General Hospital in Makassar
Dewi Kartika Tungadi, Nurhayana Sennang, Benny Rusli
.......................................................................................................................................................... 211 - 217

The Correlation of Anemia and Hepcidin Serum Levels in Regular Hemodialysis Patients with Chronic Hepatitis C
Wingsar Indrawanto, Adi Koesoema Aman, Alwi Thamrin
.......................................................................................................................................................... 218 - 223

The Comparison between Hba1c and Glycated Albumin Level Patient with Type II Diabetes Mellitus with or without CKD
M. Rusli, Zulfikar, Santi Syafril
.......................................................................................................................................................... 224 - 227

Differentiation of Thy Lymphocyte Cells Expressing Interleukin-17 on Healthy Persons and Adult Acute Myeloid Leukemia Patients
Elvan Dwi Widyadi, Yetti Hernaningsih, Endang Retnowati, Ugroseno, Ryzky Widi Atmaja
.......................................................................................................................................................... 228 - 232

LITERATURE REVIEW
Hormone Examination in Menopause
Ferdy Royland Marpaung, Trieva Verawaty Butarbutar, Sidarti Soehita
.......................................................................................................................................................... 233 - 239

CASE REPORT
Chronic Myelogeneous Leukemia Transformation into Acute Lymphoblastic Leukemia
Endah Indriastuti, Arifoel Hajat
.......................................................................................................................................................... 240 - 245

Rapid Progression of Clavicular Solitary Plasmacytoma to Multiple Myeloma
Hantoro Gunawan, Paulus Budiono Notopuro
.......................................................................................................................................................... 246 - 249
ASSOCIATION BETWEEN SPECIFIC ENOLASE SERUM LEVELS AND OUTCOME ACUTE ISCHEMIC STROKE ONE MONTH AFTER ONSET

Yuri Haiga, Darwin Amir, Yuliarni Syafrita

Department of Neurology, Andalas University, Padang, Indonesia, E-mail: yurihaiga014@gmail.com

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 marker is 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 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.5,6

The neuron-specific enolase is a dimeric iso 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. An increase 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. An increase in 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.

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.

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>
<thead>
<tr>
<th>Variable</th>
<th>Median (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58.21 (16-88)</td>
</tr>
<tr>
<td>Glasgow coma scale score at admission</td>
<td>13 ( 8-15)</td>
</tr>
<tr>
<td>Blood sugar levels at admission (mg/dL)</td>
<td>171.18 (89-402)</td>
</tr>
<tr>
<td>NSE Levels (ng/mL)</td>
<td>5.94 ( 2.77-36.75)</td>
</tr>
<tr>
<td>NIHSS score at onset &lt; 48 hours</td>
<td>6 (1-22)</td>
</tr>
<tr>
<td>mRS score at one-month after the onset</td>
<td>3 (1-6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>NSE level (ng/mL)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-45</td>
<td>11</td>
<td>4.69 (3.08 – 14.38)</td>
<td>0.24</td>
</tr>
<tr>
<td>46-88</td>
<td>66</td>
<td>6.49 (2.77 – 36.75)</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42</td>
<td>6.71 (3.31-36.75)</td>
<td>0.34</td>
</tr>
<tr>
<td>Female</td>
<td>35</td>
<td>5.67 (2.77-24.71)</td>
<td></td>
</tr>
<tr>
<td><strong>Random blood glucose level</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia (GDS &lt; 200 mg/dL)</td>
<td>20</td>
<td>6.83 (2.77-24.71)</td>
<td>0.91</td>
</tr>
<tr>
<td>Normoglycemia (GDS = 200 mg/dL)</td>
<td>57</td>
<td>5.60 (3.06-36.75)</td>
<td></td>
</tr>
</tbody>
</table>
normality by using the Kolmogorov-Smirnov test, an uneven 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 \( r = 0.286 \), 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 ischemic stroke outcome (one-month after onset) by using mRS; \( r = 0.286, p\)-value = 0.012, \( R^2 = +8.2\% \)](image)

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.\(^\text{10}\)

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.\(^\text{21}\) These results were similar with research by Brea et al., with 224 subjects studied and 69.6% males.\(^\text{12}\) 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.\(^\text{23}\) 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.\(^\text{14}\)

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 (\( p = 0.06 \)).\(^\text{15-17}\) 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.\(^\text{18}\) In this study, the ELISA technique with the Quanticle 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).^{15,22} 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.^{18,22} 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.^{21}

**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.

**Acknowledgments**

The authors want to express special thanks to all the patients who have been willing to participate in this research. Also, the authors also want to say thanks to Harry Prima, MD, Melda, MD, Dila, MD as the research team who are my fellow residents of the Department of Neurology, Faculty of Medicine Andalas University for all the help and support so that the authors could complete this research well.

**REFERENCES**