ROLE OF SERUM NEURON SPECIFIC ENOLASE (NSE) TO DIFFERENTIATE ISCHEMIC STROKE FROM HEMORRHAGIC STROKE AND ITS CORRELATION WITH BRAIN DAMAGE VOLUME

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ABSTRACT

Stroke is an emergency condition requiring immediate procedure by a neurologist. Differentiating the type of stroke (infarct or hemorrhagic stroke) and determining the extent of brain damage at the onset of the seizure is an appropriate action to determine therapy and prognosis. Increased serum NSE level in stroke can be expected to replace CT scan in differentiating stroke types at the onset of the seizure during which CT scan remains unclear and to provide assistance in determining the extent of brain damage, particularly in areas where CT Scan and MRI are unavailable. The objective of this study was to examine and compare serum NSE level in ischemic and hemorrhagic stroke patients according to lesion volume, and to analyze correlation between serum NSE level and lesion volume in CT Scan as gold standard. We examined serum NSE level of 62 acute stroke patients, comprising 31 ischemic stroke patients and 31 hemorrhagic stroke patients 24-48 (35.7 ± 8.3) hours after onset. Serum NSE level in acute stroke patients varied between 1.1 – 36 (7.45 ± 6.5) ng/ml. Serum NSE level in ischemic stroke group varied between 1.1 - 36 (6.24 ± 6.09) ng/ml, while in hemorrhagic stroke patients it varied between 1.1 – 25.3 (8.66 ± 6.76) ng/ml. CT scan lesion volume in acute stroke varied between 2.5 – 77.5 (25.5 ± 20.2) ml, in which in ischemic stroke group it was varied between 2.5 - 50.8 (15.9 ± 14.9) ml and in hemorrhagic stroke group the variation was between 7.3 -77.5 (35 ± 20.5) ml. The difference of mean serum NSE level between ischemic and hemorrhagic stroke groups was found to be insignificant (p = 0.144). Significant positive correlations in serum NSE level between ischemic stroke and CT scan lesion volume (r = +0.993; p<0.001; Pearson test), and could be used to predict brain damage volume using NSE predictive model formula = 0.406 lesion volume, and also between serum NSE level in hemorrhagic stroke and CT scan lesion volume (r = +0.894; p<0.001; Pearson test), with NSE predictive model formula = 0.294 lesion volume. The whole cases (ischemic and hemorrhagic stroke) and CT scan lesion volume had significant positive correlation (r=+0.890; p<0.001; Pearson test), with NSE predictive model = 0.286 lesion volume. In conclusion, serum NSE level in acute stroke patients (24-48 hours) after onset can be used to estimate the extent of brain damage (lesion volume), but it cannot be used to differentiate the type of stroke.

Keywords: stroke, ischemic stroke, hemorrhagic stroke, neuron specific enolase (NSE), brain damage, lesion volume

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INTRODUCTION

As an emergency condition, stroke requires immediate procedure by a neurologist. Therapy and prognosis determination, however, can be given appropriately if there is appropriate differentiation of the type of stroke (infarct or hemorrhagic stroke) and the extent of brain damage at the onset of the seizure is defined. It is believed that NSE (Neuron Specific Enolase), a dimer isoenzyme from glycolytic enolase enzyme found in nerve cells cytoplasm and neuroendocrin, can be used as a marker of brain nerve cells either in ischemic or hemorrhagic stroke. Increased NSE level immediately after brain trauma, either ischemic or hemorrhagic, was found in cerebrospinal fluid and blood.
Emergency Unit or Neurology Clinic, Dr Soetomo Hospital, and diagnosed as having acute stroke (onset 24-48 hours) with CT scan as gold standard to determine ischemic or hemorrhagic stroke and lesion volume/the extent of brain damage. Blood sample was sent to the Department of Clinical Pathology with code and time of blood taking. Results were presented as mean (+/- SD). The difference of serum NSE level between ischemic and hemorrhagic stroke was tested with independent sample $t_2$ test and correlation between serum NSE level and brain damage was tested with Pearson's correlation test.

RESULTS

A number of 66 acute stroke patients, comprising 34 ischemic stroke and 32 hemorrhagic stroke patients treated at Neurology Wards, Dr Soetomo Hospital, with onset of 24-48 hours post-stroke, were subjected to selection for sample inclusion and exclusion. Four samples (3 ischemic stroke samples and 1 hemorrhagic stroke sample) were not included due to lysis. The remaining 62 patients, 31 (50%) with ischemic stroke and 31 (50%) with hemorrhagic stroke, passed the selection and became the participants of this study. They comprised 31 women and 31 men, with age ranging from 21 to 65 years, with median of 54 years in female group and ranging from 35 to 85 years, with median of 56 years in male group.

The time of sampling was ranging between 24-48 hours after onset (early seizure), ischemic stroke group ranging between 24-48 hours (37.1 ± 7.9) hours and hemorrhagic stroke group was ranging between 24-48 hours (34.4 ± 8.6) after onset. Mean serum NSE level in ischemic group was between 1.1 – 30.6 (6.24 ± 6.09) ng/ml, while that in hemorrhagic group was higher, 1.1 – 25.3 (8.66 ± 6.76) ng/ml (Table 1). Independent sample $t_2$ test indicated no significant difference between ischemic and hemorrhagic stroke groups with $p \geq 0.05$ ($p = 0.144$). This confirmed the findings of previous authors that serum NSE level could not be used to differentiate ischemic and hemorrhagic stroke.

Lesion volume of brain damage as seen in CT scan was generally ranging between 2.5 - 77.5 (25.5 ± 20.2) ml, and in ischemic stroke the range was between 2.5 – 50.8 (15.9 ± 14.9) ml, while that in hemorrhagic stroke group was between 7.3 – 77.5 (35 ± 20.5) ml (Table 1). Pearson's correlation test revealed significant positive correlation ($p < 0.05$) between brain damage lesion volume (CT Scan) and serum NSE level in ischemic stroke group ($r = +0.993$; $p < 0.001$).

Similarly, in hemorrhagic stroke group there was significant positive correlation ($p < 0.05$) between brain damage lesion volume (CT scan) with serum NSE level in hemorrhagic stroke group ($r = +0.894$; $p < 0.001$). The whole acute stroke cases indicated significant positive correlation between brain damage lesion volume (CT scan) with serum NSE level ($r = +0.8904$ $p < 0.001$), as can be seen in Figures 1, 2 & 3. The time of sampling has confirmed the previous studies that the highest level was found on 1.9 ± 0.8 days (26.4 - 64.8 hours) after onset, as can be seen in Table 1.

Figure 1 shows significant correlation between serum NSE level and brain damage lesion volume (CT Scan) in ischemic stroke ($r = +0.993; p < 0.001$), indicating that serum NSE level can be used to predict brain damage volume in ischemic stroke by using the predictive model, which, according to regression analysis, was serum NSE level = 0.406 x lesion volume ($p < 0.001$).

Figure 2 shows significant correlation between serum NSE level and brain damage lesion volume (CT Scan) in hemorrhagic stroke ($r = +0.894; p < 0.001$), indicating that serum NSE level can be used to predict brain damage volume in hemorrhagic stroke by using the predictive model, which, according to regression analysis was serum NSE level = 0.294 x lesion volume ($p < 0.001$).

Table 1. Sample distribution to the type of stroke, range, mean NSLE level, mean lesion volume, mean sampling, and SD

<table>
<thead>
<tr>
<th>Stroke types</th>
<th>n</th>
<th>NSE range (ng/ml)</th>
<th>Mean NSE (SD) (ng/ml)</th>
<th>Lesion volume range (ml)</th>
<th>Lesion volume mean (SD) (ml)</th>
<th>Mean time of blood sampling (SD) (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic</td>
<td>31</td>
<td>1.1-36</td>
<td>6.24 (6.09)</td>
<td>2.5-50.8</td>
<td>15.9 (14.9)</td>
<td>37.1 (7.9)</td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>31</td>
<td>1.1-25.3</td>
<td>8.66 (6.76)</td>
<td>7.3-77.5</td>
<td>35 (20.5)</td>
<td>34.4 (8.6)</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>1.1-36</td>
<td>7.45 (6.5)</td>
<td>2.5-77.5</td>
<td>25.5 (20.2)</td>
<td>35.7 (8.3)</td>
</tr>
</tbody>
</table>
Predictive Model: $\text{NSE} = 0.406 \times \text{lesion volume}$

Figure 1. Serum NSE distribution in correlation with brain damage lesion volume (ST scan) in ischemic stroke.

Predictive Model: $\text{NSE} = 0.294 \times \text{lesion volume}$

Figure 2. Serum NSE level distribution in correlation with brain damage lesion volume (CT scan) in hemorrhagic stroke.

Figure 3 shows significant correlation between serum NSE level and brain damage lesion volume (CT scan) in the whole cases of acute stroke ($r = +0.894; \ p < 0.001$), indicating that serum NSE level can be used to predict brain damage volume in acute stroke by using the predictive model, which, according to regression analysis was serum NSE level = 0.286 x lesion volume ($p < 0.001$). Figures 1, 2, and 3 indicated that there was significant correlation between NSE level and brain damage lesion volume as seen in CT scan, each for ischemic stroke, hemorrhagic stroke, and whole cases (in which the higher the lesion volume, the higher the NSE serum level). This supported the study of Missler (1997) who found that infarct volume had correlation with serum NSE level.

DISCUSSION

In this study, we conducted sample examination from 31 ischemic stroke patients and 31 hemorrhagic stroke. The result indicated that serum NSE level obtained in this study was lower than that obtained in previous studies by Martens (1998) and Tiainen (2003), which was ranging between 10.4-18.8 ng/ml (mean 15.2 ng/ml) and 7.0-17.2 (mean 9.8 ng/ml) 36 hours after onset. This was possibly because those studies used different examination method with different sensitivity and specificity. Martens used immunoradiometric method, while Tiainen used immunofluorometric method.

This study used EIA sandwich immunoassay method with EIA macrophotometer, with the results similar to those of Butterworth (1996) who used spectrophotometric method, which was ranging between 5.5 ± 2.8 ng/ml in ischemic stroke and 7.5 ± 4.1 in hemorrhagic stroke. This study found NSE serum level of 6.24 ± 6.09 ng/ml in ischemic stroke and 8.66 ± 6.76 ng/ml in hemorrhagic stroke, probably because sensitivity and specificity method used by Butterworth (1996) was almost similar to the method used in this study, the EIA macrophotometer. Other studies by Schaarschmidt (1994) and Missler (1997) also showed that the result of NSE examination was higher than that.
found in studies on the release of brain damage biochemical marker, such as NSE to serum, depending on brain damage severity (Jauch 2000).

A study by Martens et al. (1998) found that increased serum Protein S 100B level is the predictive factor of regaining/not regaining consciousness in stroke patients with higher specificity and positive predictive value than serum NSE level. However, Tiainen (2003) found that serum NSE level in acute stroke is a better predictor of brain damage compared to Protein S 100B. Further studies are needed to determine which neurobiochemical marker is more sensitive and specific to determine the extent of brain damage.

Studies by Hill (2000) and Jauch (2000) revealed the importance of using other biochemical markers, such as Myelin Basic Protein (MBP), Protein S-100B, Trombomodulin (Tm), to support the diagnosis. MBP is proteolipid membrane produced by oligodendrocyte, its level in the serum and LCS increase in ischemic stroke, hemorrhagic stroke, and multiple sclerosis. Protein S-100B is a dimeric calcium-binding protein, found in astroglia and Schwann cells, whose level in serum and LCS increases in stroke, head trauma, and brain anoxia. Trombomodulin is a vascular marker, whose level increases in acute stroke. The panel of Biochemical marker examination can increase examination sensitivity up to 93% as compared to only one marker examination. In his study, Hill found that in a single examination, NSE had sensitivity of 89%, MBP 39%, Protein S-100B 32%, and Tm 43%. He wrote that in the future the examination of neurobiochemical marker panel can be used not only to differentiate the type of stroke, but also to differentiate the subtype of acute stroke.

The study by Butterworth (1996) found higher serum NSE level in hemorrhagic stroke than that in ischemic stroke. The Kruskal Wallis one-way ANOVA by ranks comparative test showed significant difference between ischemic stroke, hemorrhagic stroke, and control groups (p = 0.019). After being subjected to multiple comparative procedure, significant difference was found between hemorrhagic and control groups (p = 0.037, p < 0.05), while there was no significant difference between ischemic and hemorrhagic stroke groups p = 0.65 (p>0.05), as well as between ischemic and control groups, p = 0.098 (p > 0.05). Butterworth (1996) asserted that the one that can be used to differentiate ischemic and hemorrhagic stroke and to determine the prognosis was ratio between NSE and HSC (Human Serum Carnosinase) activity. HSC is an enzyme that hydrolizes homocarnosine, the result of the production of GABA neurotransmitter, whose activity is decreasing during acute stroke.

This study found significant positive correlation between NSE level and ischemic volume (r = +0.993 , p < 0.001), and also significant positive correlation between NSE level and hemorrhagic/bleeding volume in CT scan (r = +0.894, p < 0.001), and in general there was significant positive correlation between NSE level with lesion volume (r = +0.894, p < 0.001). This was in line with the study of Butterworth (1996) who found significant correlation between serum NSE level and the extent of brain damage, in which the highest NSE level had correlation with the widest extent of brain ischemia (Total Anterior Circulation Infarct/TACI) and the lowest level had correlation with the narrowest extent of brain ischemia (Lacunar Infarct).

This study also found similar results to those of Wunderlich (1999) and Missler (1997) who investigated correlation between serum NSE level and brain infarct volume. NIHSS score was found to have strong correlation between each other, with r = 0.69; p < 0.001 in 46 ± 6.9 hours after onset and r = 0.37, p < 0.05. This was different from the study by Schaarschmidt (1994) who found no statistical significant correlation between both, but between the prognosis and the patient's clinical condition. This study used serum sample, instead of cerebrospinal fluid, since the sampling of cerebrospinal fluid is more invasive and hazardous. Marten's study (1998) found remarkable correlation between serum NSE and cerebrospinal fluid NSE (r = 0.66; p < 0.001).

CONCLUSION

Mean serum NSE level in hemorrhagic stroke is higher (x = 8.66 ± 6.09) ng/ml from that of ischemic stroke (x = 6.24 ± 6.76) ng/ml. However, with independent sample t2 test, there was no significant correlation between serum NSE level and the type of stroke (p = 0.144), so that it cannot be used to differentiate the type of stroke. There is significant correlation between serum NSE level in ischemic stroke and CT scan lesion volume (r= +0.993; p < 0.001; Pearson's Test), and can be used to predict ischemic lesion volume with predictive model formula: NSE = 0.406 lesion volume (p < 0.001). There is significant correlation between serum NSE level in hemorrhagic stroke and CT scan lesion volume (r= +0.894; p < 0.001; Pearson's Test), and it can be used to predict hemorrhagic lesion volume with predictive model formula: NSE = 0.294 lesion volume (p < 0.001). There is significant correlation between serum NSE level in acute stroke (24-48 hours post-onset), either ischemic stroke or hemorrhagic stroke, and CT scan lesion volume (r = +0.890, p < 0.001; Pearson's Test), and can be used to predict acute stroke lesion volume with predictive model formula: NSE = 0.286 lesion volume (p < 0.001). Serum NSE
level in 24-48 hours post-onset is useful to predict the extent of brain damage and to determine patient's prognosis in areas where CT scan or MRI are unavailable.

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