Serum Neuron Specific Enolase in Hyperglycemic and Normoglycemic Ischemic Stroke Patients – A Comparative Study

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Abstract

Cerebrovascular stroke remains largely a clinical diagnosis. The use of biomarkers in diagnosing stroke and assessing prognosis is an emerging and rapidly evolving field. Neuron Specific Enolase (NSE) is mentioned as a possible reliable marker of neuronal tissue damage. It is released from neurons after infarction. High levels of serum NSE is correlated with worse clinical outcome. This study is aimed to investigate the difference in level of serum neuron specific enolase within 72 hours of stroke in hyperglycemic and normoglycemic ischemic stroke patients with left middle cerebral artery infarct and its correlation with NIHSS score & modified Rankin scale. This cross-sectional study was conducted in tertiary health care. Newly diagnosed cases of 25 hyperglycemic (FBS > 126mg/dl) and 25 normoglycemic (FBS < 126mg/dl) ischemic stroke patients (diagnosed by CT scan, clinical signs & symptoms) within the age group of 22 to 86 years of both sexes were included in the study. Serum NSE showed a positive correlation with serum FBS, NIHSS, modified Rankin scale and it was found statistically significant (P value <.0.05). The increased NSE serum levels correspond to the ischemia-induced cytoplasm loss of NSE in the neurons and are detectable before irreversible neuronal damage takes place. The rise in blood glucose levels increases serum NSE level. NSE in blood is the earliest parameter for prognostic classification, earlier than any other methods such as regularly repeated neurological examinations, neuroimaging (cranial CT or magnetic resonance imaging) or electrophysiological assays so NSE can be used as a diagnostic parameter of stroke thereby to assess stroke severity.

Keywords: Cerebrovascular stroke, Ischemic stroke, Hyperglycemia, Neuron Specific Enolase (NSE), National institutes of health stroke scale (NIHSS).

INTRODUCTION

Stroke or cerebral vascular accident, is the sudden death of brain cells due to inadequate blood flow. The WHO clinically defines stroke as “the rapid development of clinical signs and symptoms of focal neurological disturbance lasting more than 24 hours or leading to death with no apparent cause other than vascular origin [1].” Stroke is the second commonest cause of death worldwide after coronary heart disease (CHD). Cerebrovascular stroke remains largely a clinical diagnosis. The use of biomarkers in diagnosing stroke and assessing prognosis is an emerging and rapidly evolving field. Neuron-specific enolase is considered as specific neurobiochemical marker of brain damage after brain infarctions in humans [2].

NSE is released from neurons after infarction; hence, as the name implies, its specificity allows localization. After acute central nervous system insults, such as cerebral infarction, hypoxia, trauma, and seizure, the blood brain barrier is altered and astrogial disintegration substantially makes the NSE leak into cerebrospinal fluid and serum [3].

The neuron specific enolase, increases in blood circulation because it is not used anymore in damaged neuron. The half-life of NSE in serum has been reported to be about 48 hours and therefore blood levels of NSE would be expected to rise as long as infarct damage continued and NSE was washing out of brain tissue. We evaluated the serum NSE level rather than the CSF level, because the daily serum sampling was practical and posed no risk for older patients [4]. Stroke severity was a difficult variable to evaluate because of its relationship with stroke volume. Generally, larger infarcts are associated with greater stroke severity. Elevating NSE level is also connected with infarct volume and the extent of brain damage [5, 6].
Increased levels of serum NSE is correlated with worse clinical outcome [7]. Studies have shown that an initial high blood glucose concentration following a stroke is a predictor of poor outcome [8]. A high proportion of patients suffering an acute stress such as stroke may develop hyperglycemia even in the absence of a pre-existing diagnosis of diabetes [9]. Hyperglycemia defined as fasting blood glucose >126mg/dl [10] is correlated with elevated levels of NSE and the NIHSS score. Hyperglycemia may be directly toxic to the ischemic brain. Accumulation of lactate and intracellular acidosis in the ischemic brain (produced through anaerobic cerebral glucose metabolism) may contribute. Intracellular acidosis may promote and accelerate ischemic injury by enhancing lipid peroxidation and free radical formation [11], allowing accumulation of intracellular calcium [12] (a key component of the glutamate-dependent excitotoxicity seen in ischemic neurons) [13], and impairing mitochondrial function [14]. These neurotoxic effects may be particularly important in the ischemic penumbra [15]. The lipid profile also might have an important role in those ischemic strokes that are the consequence of atherosclerosis of larger arteries.

The aim of this cross-sectional study is therefore to compare serum neuron specific enolase levels in hyperglycemic and normoglycemic ischemic stroke patients with left middle cerebral artery infarct since neuron specific enolase is a marker of neuronal tissue damage and a prognostic parameter in cerebrovascular disease. Assessment of stroke severity is done by NIHSS score and modified Rankin scale in our study.

AIMS AND OBJECTIVES

AIMS
To find out difference in serum neuron specific enolase level in hyperglycemic and normoglycemic ischemic stroke patients.

OBJECTIVES
• To correlate serum neuron specific enolase with NIHSS score and modified Rankin scale
• To study the variation of lipid profile level in hyperglycemic and normoglycemic ischemic stroke patients.

MATERIALS AND METHODS

It is a Hospital based cross-sectional study conducted in a tertiary health care. 25 hyperglycemic and 25 normoglycemic ischemic stroke patient with informed consent are included for the study after approval of institutional ethical committee.

Inclusion Criteria
• Newly diagnosed cases of hyperglycemic and normoglycemic ischemic stroke patients within the age group of 22 to 86 years of both sex.
• hyperglycemic ischemic stroke -diagnosed by CT scan, clinical signs & symptoms & FBS > 126mg/dl
• normoglycemic ischemic stroke - diagnosed by CT scan, clinical signs & symptoms & FBS < 126mg/dl

Exclusion Criteria

Patients with stroke of more than 72 hours, central nervous system infections, peripartum stroke, head trauma, central nervous system, tumors, seizures.

About 5 ml of blood was drawn after 12 hours of fasting using disposable syringes and needles under strict aseptic precautions, from cubital vein and collected in a test tube. Serum NSE, fasting blood glucose, Fasting lipid profile were analyzed. Serum NSE was estimated by enzyme immunoassay based on biotin double antibody sandwich technology. Blood glucose was assayed by the glucose oxidase method. Total cholesterol, HDL and TGL were estimated by CHOD-PAP method, Direct method and Enzymatic method respectively. FRIEDEWALDS formula was used to calculate LDL = TC – (HDL + TGL / 5).

ANALYSIS AND RESULTS

Statistical analysis was performed using SPSS for windows version 17. The mean and standard deviation for quantitative variables and percentage for qualitative variables were calculated for 25 hyperglycaemic and 25 normoglycemic ischemic stroke patients. Chi square test was used to compare differences in the percentage of qualitative variables between the groups. Differences in means of quantitative variables between the two groups were compared by student t test. P value of less than 0.05 is considered significant. Pearson correlation coefficient was obtained to study correlation between serum NSE, fasting blood glucose and lipid profile parameters.
Table 1: Comparison of test parameters between hyperglycaemic and normoglycemic ischemic stroke patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Stroke patients</th>
<th>n</th>
<th>Mean</th>
<th>S.D</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS</td>
<td>Hyperglycaemic</td>
<td>25</td>
<td>215.6</td>
<td>64.1</td>
<td>8.016</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Normoglycaemic</td>
<td>25</td>
<td>109.0</td>
<td>17.6</td>
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<td></td>
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<tr>
<td>NSE</td>
<td>Hyperglycaemic</td>
<td>25</td>
<td>13.5</td>
<td>5.0</td>
<td>4.361</td>
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<tr>
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<td>25</td>
<td>8.8</td>
<td>2.2</td>
<td></td>
<td></td>
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<tr>
<td>Modified Rankin</td>
<td>Hyperglycaemic</td>
<td>25</td>
<td>3.8</td>
<td>.5</td>
<td>8.017</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>scale</td>
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<td>25</td>
<td>2.6</td>
<td>.5</td>
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</tr>
<tr>
<td>NIHSS score</td>
<td>Hyperglycaemic</td>
<td>25</td>
<td>13.2</td>
<td>5.2</td>
<td>5.633</td>
<td>&lt;0.001</td>
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<td></td>
<td>Normoglycaemic</td>
<td>25</td>
<td>6.4</td>
<td>2.9</td>
<td></td>
<td></td>
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<tr>
<td>Total cholesterol</td>
<td>Hyperglycaemic</td>
<td>25</td>
<td>203.5</td>
<td>38.3</td>
<td>3.294</td>
<td>.002</td>
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<tr>
<td></td>
<td>Normoglycaemic</td>
<td>25</td>
<td>169.2</td>
<td>35.3</td>
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<td></td>
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<tr>
<td>HDL</td>
<td>Hyperglycaemic</td>
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<td>37.9</td>
<td>7.1</td>
<td>-.831</td>
<td>.410</td>
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<td>25</td>
<td>40.2</td>
<td>11.8</td>
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<td>TAG</td>
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<td>125.4</td>
<td>48.1</td>
<td>1.262</td>
<td>.213</td>
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<tr>
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<td>25</td>
<td>109.7</td>
<td>39.6</td>
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<td></td>
</tr>
<tr>
<td>LDL</td>
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<td>140.6</td>
<td>37.7</td>
<td>3.222</td>
<td>.002</td>
</tr>
<tr>
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<td>Normoglycaemic</td>
<td>25</td>
<td>107.1</td>
<td>35.7</td>
<td></td>
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</tr>
</tbody>
</table>

(FBS- Fasting blood sugar, HDL- High density lipoprotein, LDL- Low density lipoprotein, NSE- Neuron Specific Enolase, NIHSS – National institutes of health stroke scale).

Fig 1: Mean value of FBS in study groups

The mean FBS values among hyperglycaemic stroke patients were 215.6 mg/dl and among normoglycaemic stroke patients were 109. The difference was found to be statistically significant (P value < 0.05).

Fig 2: Mean value of NSE, Modified Rankin Scale and NIHSS in study groups

The mean serum NSE level was 13.5 among hyperglycaemic stroke patients while it was 8.8 among normoglycaemic stroke patients. This difference was found to be statistically significant (P value < 0.05). The mean NIHSS score was 13.2 among hyperglycaemic stroke patients while it was 6.4 among normoglycaemic stroke patients. This difference was found to be statistically significant (P value <.05). The mean modified Rankin scale was 3.8 among hyperglycaemic stroke patients while it was 2.6 among normoglycaemic stroke patients. This difference was found to be statistically significant (P value <.05).

Fig 3: Mean value of lipid profile in study group

The mean serum cholesterol was 203.5 among hyperglycaemic stroke patients while it was 169.2 among normoglycaemic stroke patients. This difference was found to be statistically significant (P value <.05). The mean serum Triacylglycerol was 125.4 among hyperglycaemic stroke patients while it was 109.7 among normoglycaemic stroke patients. This difference was found to be statistically insignificant (P value >.05). The mean serum LDL was 140.6 among hyperglycaemic stroke patients while it was 107.1 among normoglycaemic stroke patients. This difference was found to be statistically insignificant (P value >.05).
Table-2: Pearson correlation between test parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pearson Correlation (r)</th>
<th>P</th>
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<tbody>
<tr>
<td>glucose</td>
<td>.585**</td>
<td>&lt;0.001</td>
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<tr>
<td>Modified Rankin scale</td>
<td>.637**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NIHSS</td>
<td>.763**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>.200</td>
<td>.163</td>
</tr>
<tr>
<td>HDL</td>
<td>-.019</td>
<td>.894</td>
</tr>
<tr>
<td>TAG</td>
<td>-.036</td>
<td>.806</td>
</tr>
<tr>
<td>LDL</td>
<td>.214</td>
<td>.136</td>
</tr>
</tbody>
</table>

Serum NSE showed a positive correlation with modified Rankin scale and it was found statistically significant (P value <.05).

DISCUSSION

A major proportion of stroke patients have stress hyperglycemia which increases the neuronal damage. The present study deals with the role of NSE as a marker of neuronal damage in ischemic stroke patients thereby comparing the severity of damage between hyperglycemic and normoglycemic ischemic stroke patients. Severity of neuronal damage was assessed by correlating NSE level with NIHSS score and modified Rankin scale. The increased NSE serum levels correspond to the ischemia-induced cytoplasm loss of NSE in the neurons and are detectable before irreversible neuronal damage takes place [16]. The mean serum NSE level among hyperglycemic ischemic stroke patients was 13.5± 5.0 and in normoglycemic ischemic stroke patients was 8.8 ± 2.2. Student t test was done to analyse the difference in mean NSE levels between these groups and the difference was found statistically significant. Pearson correlation was done to analyse the correlation between serum NSE and FBS levels. There was strong positive correlation between these two variables (r = 0.585). This shows that as FBS levels increase, NSE levels increase. This was in accordance with the study by Pandey et al., in 2011 which had shown raised serum NSE level among hyperglycemic ischemic stroke patients [17].

NSE level shows strong positive correlation with NIHSS score (r= 0.763) and modified Rankin scale (0.637). These results was comparable with the study of Kiers L, Davis SM et al who have concluded hyperglycemia appears to be associated with more severe stroke, as assessed with a clinical stroke scale [18]. Our study is also comparable to studies reported by Selakovic V et al., [4] and Myers et al., [19]. There was no significant difference in the distribution of NSE levels in accordance with age or sex among the two groups. This was in accordance with Missler et al., who showed that the serum level of NSE was not related with age or sex [6].

Various lipid profile parameters like serum total cholesterol, triglyceride, HDL and LDL were compared between hyperglycemic and of the normoglycemic ischemic stroke patients. There was no statistically significant relation on doing chi square test. NSE level has positive correlation with serum cholesterol (r= 0.200) and LDL level (r = 0.214). NSE level has negative correlation with TAG (r = -0.36) and HDL level (-0.19). The mechanism of lipid changes remains unclear, but it is thought to relate in part to the stress and associated catecholamine overproduction of an acute stroke.
CONCLUSION

NSE in blood is the earliest parameter for prognostic classification, earlier than any other methods such as regularly repeated neurological examinations, neuroimaging (cranial CT or magnetic resonance imaging) or electrophysiological assays. Serum NSE levels are high in hyperglycaemic ischemic stroke patients compared to normoglycemic ischemic stroke patients. Neuron specific enolase (NSE) is a reliable marker of neuronal damage. So NSE can be used as a diagnostic parameter of stroke thereby to assess stroke severity.

REFERENCES