High serum S-100B protein levels within the first 36 hours of acute ischemic stroke predicts high NIHSS scores

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Abstract
Aim: The aim of the present study was to evaluate serum levels of S-100B which is an acidic calcium-binding protein within the first 36 hours following an acute ischemic stroke and to determine the correlation between S-100B levels and National Institutes of Health Stroke Scale (NIHSS) scores.

Material and Methods: Fifty patients with ischemic stroke (25 male, 25 female; mean age=64) and 30 healthy volunteers (14 male, 16 female; mean age=61) serving as age and sex matched controls were included in this study. Venous blood samples were taken within the first 36 hours following the acute stroke. The quantitative determination of S-100B in serum was carried out using an enzyme linked immunosorbent assay.

Results: Patients with acute ischemic stroke had significantly higher serum S-100B protein concentrations (42 pg/ml) than those of normal controls (31.5 pg/ml) (p<0.001). S-100B concentrations were correlated with NIHSS scores (r=0.443; p=0.001), which is considered an indicator of the degree of neurological deficit.

Conclusion: The present study demonstrates that S-100B concentrations in both the large artery atherosclerosis (LAA), cardioembolic infarct (CEI) subtypes of acute stroke are increased in the early phases of stroke and are correlated with NIHSS scores upon admission. S-100B appears to be a valuable biomarker of ongoing brain injury.

Keywords: Cerebral infarction; stroke; S-100B Protein; S-100B Calcium Binding Protein beta Subunit

INTRODUCTION
Inflammatory mechanisms are involved in brain injury and blood brain barrier (BBB) breakdown in acute ischemic stroke. Some proteins secreted by astrocytes after brain injury act like inflammatory mediators and contribute in progressive brain damage. These proteins enter cerebrospinal fluid (CSF) and bloodstream through deranged BBB, therefore they are regarded as biomarkers for brain injury (1,2). Previous studies evaluated the sensitivity and specificity of various biomarkers in stroke. These proteins are mainly CSF markers for cerebral infarction which leads to increased levels in response to injury associated with the extent of ischemic edema. However spinal tap is an invasive procedure and diagnostic yield of routine CSF assessment is low in ischemic stroke. Therefore the clinical use of blood-based biomarkers such as S-100B which reflects ischemic injury in stroke has been evaluated (3). S-100B, which is an acidic calcium-binding protein located in astrocytes, could accumulate in the extracellular space following the secretion from damaged cells. Therefore, S-100B penetrates both the blood and cerebrospinal fluid (CSF) which may lead to increased inflammation (4). S-100B contributes in intracellular functions such as modulation of cytoskeleton proteins, regulation of cellular cytokines and extracellular functions depending on the S-100B levels (5). Furthermore, S-100B can operate as a valuable blood based biomarker of ischemic stroke reflecting clinical severity of neurological status (5,6).

The aim of the present study was to evaluate serum levels of S-100B within the first 36 hours following an acute ischemic stroke and to determine whether these levels correlate with neurological status assessed by National Institutes of Health Stroke Scale (NIHSS) scores.
MATERIAL and METHODS

This prospective cohort study was conducted among patients with acute ischemic stroke who admitted Gazi University Faculty of Medicine- Ankara over a 13 month period. The study was approved by the local ethics committee and informed consent for participation was obtained from each participant or from their relatives. A total of 50 patients (25 male, 25 female; mean age=64±15) with acute ischemic stroke were included in the study. Age and sex matched 30 healthy volunteers (14 male, 16 female; mean age=61±12) served as controls. Healthy controls without trauma, systemic or neurological diseases were included. Neurological deficits were measured upon admission by a trained neurology resident employing the National Institutes of Health Stroke Scale (NIHSS) (7). Stroke was verified by neuroimaging in patients who presented with focal neurological deficits which are believed to be caused by a vascular lesion which has lasted more than 24 hours. Ischemic stroke was classified according to the Oxfordshire scale (8). Cardiovascular risk factors such as type 2 diabetes, hypertension, atherosclerotic heart diseases, cerebrovascular disease, hyperlipidemia, smoking and atrial fibrillation were evaluated both for patients with stroke and in control subjects.

The type of acute ischemic stroke was classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification: 1) large artery atherosclerosis (LAA), 2) cardioembolic infarct (CEI), 3) lacunar infarction (LAC), 4) stroke of other determined etiology (ODE), and 5) stroke of undetermined etiology (UDE) (9). Only patients with LAA, CEI ischemic stroke subtype were included to the study because of the extremely small size of the LAC, ODE and UDE subgroups.

S-100B Determination

Blood samples were collected within the first 36 hours following the acute ischemic stroke. The samples were taken by a nurse using routine vein puncture with 5 ml syringes to the laboratory tubes and they were immediately centrifuged (3000 g, 5 minutes), frozen at 85°C, and kept in the freezer (Ultra Low Freezer; NUAIRE) until analysis. The quantitative determination of serum S-100B was carried out using an enzyme linked immunosorbent assay (ELISA; BioVendor; Heidelberg, Germany) on samples according to the manufacturer’s kit instructions.

Statistical evaluation

Statistical analyses were performed using the SPSS 15 software package (SPSS Inc.). The Shapiro-Wilk test was used to determine whether numeric variables showed a normal distribution. For those that showed a normal distribution, the mean and standard deviation were used (x± SD). If the variables did not show a normal distribution, median (min-max) values were determined when two groups were compared. When the age variable was compared between two groups t-test and a Mann-Whitney U test was utilized if S-100B levels did not have a normal distribution. When the type of stroke was compared among three groups, a Kruskal-Wallis test was utilized if S-100B levels did not have a normal distribution. The correlation between S-100B and NIHSS scores was determined using Spearman’s correlation coefficient with a p value less than 0.05 considered statistically significant.

RESULTS

The LAA group consisted of 36 patients (20 male, 16 female; mean age=60±16) and the CEI group had 14 patients (5 male, 9 female; mean age=70±11). NIHSS score of the patients was 10±5 (2-26). Patients with acute ischemic stroke had significantly higher S-100B protein concentrations: 42 pg/ml (3-2600) than those of normal controls: 31.5pg/ml (3-82); this difference (p<0.001) was independent of age and sex (Table 1). No significant differences were present in between serum S-100B levels in male and female groups both in patients (p=0.517) and in control subjects (p=0.961).

Additionally, S-100B protein concentrations were significantly higher in the LAA group: 41 ng/ml (24-2600) and CEI group: 50 pg/ml (3-1000) relative to controls: 31.5 pg/ml (3-82) (p=0.002) (Figure 1). No statistically significant difference was present between the S-100B levels of the LAA and CEI subtypes of acute ischemic stroke (Table 2). Regarding risk factors such as hypertension, diabetes mellitus, hyperlipidemia, smoking history, coronary artery disease, atrial fibrillation, and cerebrovascular disease history, there were no statistically significant differences were present in S100B protein levels between the two patient groups.

Table 1. Characteristics, S-100B values and NIHSS of patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n=30)</th>
<th>LAA (n=36)</th>
<th>CEI (n=14)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>61.6 ± 12.2</td>
<td>60.0 ± 16.9</td>
<td>70.0 ± 11.5</td>
<td>0.164</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 (46.7%)</td>
<td>20 (55.6%)</td>
<td>5 (35.7%)</td>
<td>0.434</td>
</tr>
<tr>
<td>Female</td>
<td>16 (53.3%)</td>
<td>16 (44.4%)</td>
<td>9 (64.3%)</td>
<td></td>
</tr>
<tr>
<td>S-100B (pg/ml)</td>
<td>31.5 (3-82)</td>
<td>41 (24-2600)</td>
<td>50 (3-1000)</td>
<td>0.002</td>
</tr>
<tr>
<td>NIHSS</td>
<td>95 (2-26)</td>
<td>116 (2-24)</td>
<td></td>
<td>0.362</td>
</tr>
</tbody>
</table>

LAA: Large Artery Atherosclerosis; CEI: Cardioembolic Infarct; NIHSS: National Institutes of Health Stroke Scale
Figure 1. Levels of S-100B in large artery atherosclerosis, cardioembolic stroke and in normal controls

Table 2. S-100B (pg/ml) values grouped according to risk factors in patients with acute ischemic stroke

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>N</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Z</th>
<th>p</th>
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</thead>
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<tr>
<td>Diabetes</td>
<td>-</td>
<td>37</td>
<td>42</td>
<td>3</td>
<td>2600</td>
<td>-0.044</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>13</td>
<td>45</td>
<td>4</td>
<td>2400</td>
<td>-0.044</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-</td>
<td>19</td>
<td>42</td>
<td>25</td>
<td>2600</td>
<td>-1.240</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>31</td>
<td>42</td>
<td>3</td>
<td>1000</td>
<td>-0.044</td>
</tr>
<tr>
<td>Atrial Fibrillation</td>
<td>-</td>
<td>42</td>
<td>41</td>
<td>3</td>
<td>2600</td>
<td>-0.781</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>8</td>
<td>50</td>
<td>27</td>
<td>1000</td>
<td>-0.044</td>
</tr>
<tr>
<td>Atherosclerotic Heart Disease</td>
<td>-</td>
<td>47</td>
<td>42</td>
<td>4</td>
<td>2600</td>
<td>-1.124</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>3</td>
<td>39</td>
<td>3</td>
<td>43</td>
<td>-0.044</td>
</tr>
<tr>
<td>Cerebrovascular Disease</td>
<td>-</td>
<td>41</td>
<td>43</td>
<td>24</td>
<td>2600</td>
<td>-1.327</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>9</td>
<td>36</td>
<td>3</td>
<td>1000</td>
<td>-0.044</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>-</td>
<td>35</td>
<td>43</td>
<td>3</td>
<td>2600</td>
<td>-0.392</td>
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<tr>
<td></td>
<td>+</td>
<td>15</td>
<td>40</td>
<td>24</td>
<td>1000</td>
<td>-0.044</td>
</tr>
<tr>
<td>Smoking</td>
<td>-</td>
<td>42</td>
<td>41</td>
<td>3</td>
<td>2400</td>
<td>-1.284</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>8</td>
<td>50</td>
<td>36</td>
<td>2600</td>
<td>-0.044</td>
</tr>
</tbody>
</table>

NIHSS: National Institutes of Health Stroke Scale

Figure 3. Correlation of S-100B with NIHSS in group with large artery atherosclerosis

Figure 4. Correlation of S-100B with NIHSS in group with cardioembolic infarction
Of the study participants, eight patients (16%) were current smokers and the other risk factors were as follows, respectively: hypertension (31, 62%), hyperlipidemia (15, 30%), diabetes (13,26%), atrial fibrillation (8,16%), history of cerebrovascular disease (9,18%), atherosclerotic cardiac disease (3,3%). Differences in S-100B values were evaluated according to the risk factors but did not significantly differ in this respect (Table 2). S-100B concentrations were correlated with NIHSS scores (r=0.447; p=0.001), which is considered an indicator of the degree of neurological deficit. (Figure 2,3,4).

DISCUSSION

Biochemical indicators of brain injury has attracted great attention for many years. Molecular indicators affiliated with stroke are considered to be valuable tools for diagnostic evaluation (2). Biochemical indicators of neurotoxicity and inflammation have been associated with early neurological worsening, extent of ischemic lesion volume as well as hemorrhagic transformation of ischemic regions (10,11). Traditionally; S-100B has been accepted as an accurate indicator of astrocytic brain injury. It has been demonstrated that S-100B is localized in particular cell types such as neurons, astrocytes, oligodendrocytes as well as in choroid plexus epithelium (12). Rather than showing definitive glial damage S-100B has been implicated as an indicator of BBB injury (13). S-100B is also present in extraneural tissues like adipocytes, chondrocytes, melanocytes but transition from these tissues to blood is unclear (14,15). Cerebrospinal fluid concentrations of S-100B are 40 times higher than in serum concentrations. Due to impaired BBB; spill of S100-B from cerebrospinal fluid leads to movement of S-100B from cerebral tissue to vascular space leading to quick rise in serum concentrations (11). Pathophysiologically, release of S-100B into serum in acute ischemic injury is thought to demonstrate astroglial cell death which leads to leakage of this marker because of deranged BBB (3). Increased levels of serum S100-B may also be due to presence of both cytotoxic and vasogenic edema (6). Highest levels are present within 2 to 4 days in animals with middle cerebral artery occlusions whereas S100-B achieved its highest values at about 7.5 hours in animals with brain contusion (16). In accordance with experimental studies a number of clinical studies have showed increased values as of serum S-100B in patients with acute cerebral infarction (3,13,17-25). These studies found that S-100B levels are increased with in the first 3 days of an acute ischemic injury and that patients with large artery cortical infarcts exhibit higher serum S-100 values as compared to patients with lacunar infarcts (3,18,19,23) . Following symptom onset there is a steady increase in S100-B beginning at 8-10 h, peaking at 72h with a gradual decrease at 96h (13). Samples gathered more than 24h following symptom onset revealed a close relationship with the extent of neurological disability as well as with final ischemic lesion volume (11,18-20,23,24). It has been demonstrated that samples taken with in the first 2 hour of symptom onset did not correlate with initial NIHSS scores, early ischemic lesions on brain CT and neither with lesion volume (13,25). However S-100B values which were gathered beyond 24 h following symptom onset revealed a good association with the final infarct volume (3,17-22). S-100B levels measured in geriatric patients with in the first, 3rd and 14th day of acute ischemic stroke revealed that increased levels of S-100B with in the first and 3rd day of acute stroke correlated with poor outcome (26). In the present study, we evaluated serum S-100B levels within the first 36 hours of acute ischemic stroke in order to determine the time period which S-100 B increases, because in the previous studies the evaluations were performed usually within the first 24 or 72 hour (3,13,19,23). We chose a midline time point in order not to duplicate the previous data. It is believed that S-100B is liberated from necrotic astroglial cells following cerebral ischemia. Data from patients with recanalised cerebral arteries demonstrated that; patients with small final infarct volume did not acquire increased serum S-100B levels (13). S-100B protein levels were significantly higher in patients with multiple arterial territorial infarcts, followed by isolated middle, anterior and posterior cerebral infarctions; respectively (27).

The present study demonstrates that S-100B concentrations in both the LAA and CEI subtypes of acute stroke are increased in the early phases of stroke and are associated with stroke severity as assessed using the NIHSS upon admission.

The correlation between increased S-100B levels and neurological disability in the acute state of ischemic stroke is debatable because some studies declined to reveal that (19,27) whereas the others showed the correlation (13,20,28). The inconsistency that are present in these studies may be due to extended and slow release of these biomarkers into the blood in the acute state and management of sample collection which is not standardized in most of the studies (29). S-100B which has a short half-life bears a close relationship with brain injury as well as with neurological disability and outcome. It is not affected by patients age, sex or with the presence of concomitant systemic disease (29). When levels of S-100B are evaluated in timely intervals the changes in serum levels may predict the neurological course when other indicators are not accessible or achievable (14).

Cardioembolic strokes are known to be associated with higher stroke severity and lower collateral circulation leading to greater volume of ischemic infarct (30,31).

In our study S-100B levels were strongly correlated with NIHSS scores among patients with CEI rather than patients with LAA. Although we did not obtain ischemic infarct volume on MRI, this correlation could account for the greater volume of ischemic infarct in CEI associated with higher S-100B levels.
There were some limitations of the present study. Primarily, the sample size of the study was small and sequential measurements of S-100B were not performed. We were not able to include all subtypes of ischemic stroke groups according to TOAST classification because of extremely small size of the other subgroups. S-100B which is also present in extra-neural tissues may influence serum S-100B levels. Although healthy controls were screened for systemic and neurological diseases, they didn’t go under further evaluation. CSF levels of S-100B were not acquired in the present study in order to compare CSF and serum levels of S-100B because of invasiveness of the procedure.

CONCLUSION
The present study revealed increased serum S-100B levels within the first 36 hours of acute ischemic stroke. We believe these results demonstrate that S-100B protein which is released from the damaged brain tissue disseminates from CSF to blood via impaired BBB in the early phases of ischemic stroke. In the present study, no association in the levels of S-100B and the etiological cause of ischemic stroke were present. Serum S-100B levels correlated with NIHSS in both LAA and CEA subtypes of stroke. S-100B can be used as a valuable biomarker in order to determine the extent of brain injury in the early phase of ischemic stroke. These results need to be replicated with further studies in larger sample size of stroke patients in order to confirm and settle this issue.

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Competing interests: The authors declare that they have no competing interest.

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Ethical approval: The study was approved by Gazi University Faculty of Medicine ethics committee (2007-246 protocol number).

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