ABSTRACT

Background: Traumatic brain injury (TBI) is a common cause of death and disability, worldwide. Early recognition of patients with brain cellular damage allows for early rehabilitation and patient outcome improvement. Serum protein S-100B determinations have been widely suggested the most promising biomarker for TBI. It has been proposed that this marker is useful in a Neurointensive Care Unit (NICU) as a monitoring parameter. The main objective of this study is to assess the value of including acute S100B levels in standard clinical data as an early screening tool for brain death after severe TBI. Material and methods: In this prospective study, the clinical conditions of patients with mild to moderate TBI were assessed and patient serum S100B levels measured within 24 h of injury were eligible for inclusion in the study using electrochemiluminescence (ECL). Patients were admitted to The Govt. Trauma Centre, P.B.M. Hospital, Bikaner in NICU and followed up one month later and evaluated for level of consciousness, presence or absence of post-traumatic headache, and daily activity performance (using the Barthel scale). Student’s t-test and the chi-square test were used for the data analysis, which was performed using SPSS software. Result and discussion: The mean serum S100B value was significantly lower for patients with minor TBI than for patients with moderate TBI (20.4 ± 12.6 ng/dl and 124.0 ± 235.0 ng/dl, respectively). Patients with normal CT scans also had statistically significantly lower serum S100B levels than patients with abnormal CT findings. The mean S100B value was statistically significantly higher for patients with suspected diffused axonal injury (596.18 ± 502.1 ng/dl) than for patients with other abnormal CT findings (p = 0.000): 20.97 ± 19.9 ng/dl in patients with normal CT results; 39.56 ± 21.7 ng/dl in patients with skull bone fracture; 50.38 ± 22.9 ng/dl in patients with intracranial haemorrhage; and 70.23 ± 31.3 ng/dl in patients with fracture plus intracranial haemorrhage. Conclusion: Serum S100B levels increase in patients with minor to moderate TBIs, especially in those with diffused axonal injury. However, serum S100B values cannot accurately predict one-month neuropsychological outcomes and performance.

KEYWORDS: Skull bone fracture, Axonal injury, Haemorrhage, Neuropsychological, SPSS Software, Post-traumatic Headache, Abnormal CT

INTRODUCTION

Prognosis of patient outcome after traumatic brain injury (TBI) primarily incorporates clinical predictors, such as age, pupillary reactivity, motor score, and computerized tomography (CT) characteristics; these variables only provide moderate sensitivity and specificity for accurate prognosis [1], [2]. Additional studies suggest that predictive models, built using large
populations with TBI, vary in their applicability to other populations. Predictive models are also subject to variation, based on covariate utilization [3] and incorporation of baseline characteristics [1]. The heterogeneity involved with TBI mechanism, injuries, treatments, and recovery patterns may contribute to this issue [4]. The incorporation of bio markers into predictive outcomes models may be one approach to address this heterogeneity.

Proteomic biomarkers are a part of mainstream clinical care used to quantitatively assess and define injury in almost every organ system, except the brain [5], [6]. With the absence of specific prognostic tools, there is increasing interest in identifying biomarkers that are both sensitive and specific to the central nervous system (CNS) to aid in diagnosis and prognosis for individuals sustaining TBI [6].

S100B, a calcium binding protein highly expressed in astroglial cells of the brain and released in cerebrospinal fluid (CSF) and blood, can be measured by available immunoassay kits. Different studies have evaluated S100B as a biomarker for different brain injuries, such as stroke [7], [8], bacterial meningitis [9], carbon monoxide poisoning [10], and TBI [11]–[14]. Some recent studies have also highlighted the complex release pattern of S100B and its potential role in brain tissue repair processes [15]–[19]. This prospective study evaluates the diagnostic and prognostic roles of serum S100B protein in emergency department (ED) patients with minor to moderate TBI.

MATERIALS AND METHODS

Patients were enrolled conveniently between January and August 2015 at The Govt. Trauma Centre, P.B.M. Hospital, Bikaner in Neuro-intensive care unit (NICU) and followed up one month later and evaluated for level of consciousness, presence or absence of post-traumatic headache, and daily activity performance of 200 adult patients and S.P. Medical College, Bikaner, approved this prospective study, and informed consent was obtained from all patients.

Participants

Patients at least 18 years old with a clinical diagnosis of acute mild to moderate TBI were enrolled. Patients with a history of isolated head trauma and Glasgow coma scale (GCS) score between 9 and 15 who presented in the ED within the first six hours of their head injury were considered to have mild to moderate TBI. All clinical assessments, including GCS calculations, were performed by a research assistant who was a physician. The research assistant was blinded to other assessments results.

Patients with the following were excluded: severe TBI (GCS<8); hemodynamic instability; body temperature greater than 38.5°C; concurrent trauma to any other organs; concurrent primary and secondary brain injury, including refractory severe hypoxia (arterial oxygen saturation <92% while receiving 100% oxygen), post-traumatic seizure, and skull bone fracture; and any other identified or suspected differential diagnosis for the patient’s decreased level of consciousness, including alcohol abuse, drug abuse, substance abuse, drug toxicity, hypo/hyperglycaemia, hypo/hypernatremia, endocrine disorder, or infection. Patients who did not undergo a head CT scan were also excluded (Table 1).

Intervention

S100B assay: A blood sample was drawn from the peripheral veins within the first six hours of ED admission. The time of blood sample collection was recorded. Samples were centrifuged and the serum was refrigerated at –20°C until analysed. Neuroimaging: Ten millimetre thick slices obtained using a GE VCT light speed 64 multi slice detector were interpreted by a board certified radiologist and confirmed by another consultant radiologist who was blinded to the first interpretation.

Both radiologists were blinded to the clinical conditions and S100B results of the patients. All pathologic findings, including skull bone fracture and any type of intracranial haemorrhage (e.g., brain contusion,
subdural/epidural intracranial hematoma), were reported as positive computed tomography findings.

Follow up: The patients were called by two blinded research assistants one month later. During follow-up, patients were evaluated for level of consciousness, presence or absence of post-traumatic headaches, and daily activity performance (using the Barthel scale) to determine if any significant intracranial complications had occurred (i.e., complications requiring further neuroimaging).

Measurements

Initial TBI severity was assessed using the GCS. Patients with GCS scores between 9 and 15 were considered to have mild to moderate TBI. To measure S100B serum levels, the humanS100 ELISA kit (Bio Vendor - laboratorni medicina a.s., Brno, Czech Republic) was used. The lowest detection limit of the test is about 15 pg/ml. Serum S100B levels were measured in ng/dl.

The Barthel scale is an ordinal 10-variable scale used to measure patient performance on daily activities and to predict the likelihood a patient will be able to live at home independently.

The Barthel scale has high inter-rater and test retest reliability, as well as, high correlations with other measures of physical disability. The ten Barthel scale variables are: presence/absence of faecal incontinence; presence/absence of urinary incontinence; and help needed with grooming, toilet use, feeding, transfers, walking, dressing, climbing stairs, and bathing. Each variable is given a score (between 0 and 3). These scores are summed to determine the total score (out of 20). The higher the Barthel score, the less assistance the patient is likely to need with daily activities after discharge from the hospital. For example, when a person can perform about 50% of their daily tasks and activities independently, then their Barthel score will be 10 out of 20 [20]–[22]. Patient outcome measures were level of consciousness, residual headache, and Barthel score one month after trauma.

Data Analysis

The Student’s $t$-test was used to compare the mean values of quantitative variables, and the Chi square test was used to compare qualitative variables. All data analyses were performed with SPSS version 13.5 (SPSS, Inc., Chicago, IL).

RESULTS

Two hundred patients were assessed for eligibility, and 82 patients were excluded from the study: six patients had hemodynamic instability; 22 patients had concurrent trauma to other organs; 38 patients had concurrent brain injuries; and 18 patients had other causes of decreased level of consciousness. Venous blood samples were obtained from 118 patients with minor to moderate TBI who had undergone CT as a part of their routine diagnostic evaluations. Two samples were wasted due to various errors between initial preparation and analysis. A total of 108 patients with mild to moderate TBI and available serum S100B results were followed.

### Table 1: Basic characteristic of study participants

<table>
<thead>
<tr>
<th>Category</th>
<th>Variable</th>
<th>Number (n)</th>
<th>Per cent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>102</td>
<td>86.44</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>16</td>
<td>13.55</td>
</tr>
<tr>
<td>Initial GCS</td>
<td>15</td>
<td>78</td>
<td>66.10</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2</td>
<td>0.01</td>
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<tr>
<td></td>
<td>13</td>
<td>32</td>
<td>27.11</td>
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<tr>
<td></td>
<td>12</td>
<td>19</td>
<td>16.10</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>12</td>
<td>10.16</td>
</tr>
<tr>
<td>Mechanism of Injury</td>
<td>Auto pedestrian</td>
<td>46</td>
<td>38.98</td>
</tr>
<tr>
<td></td>
<td>MVC</td>
<td>30</td>
<td>25.42</td>
</tr>
<tr>
<td></td>
<td>Falling</td>
<td>29</td>
<td>24.57</td>
</tr>
<tr>
<td></td>
<td>Direct trauma</td>
<td>19</td>
<td>16.10</td>
</tr>
<tr>
<td>CT Finding</td>
<td>Normal</td>
<td>68</td>
<td>57.62</td>
</tr>
<tr>
<td></td>
<td>DAI</td>
<td>9</td>
<td>07.62</td>
</tr>
<tr>
<td></td>
<td>ICH</td>
<td>12</td>
<td>10.16</td>
</tr>
<tr>
<td></td>
<td>fx</td>
<td>15</td>
<td>17.7</td>
</tr>
</tbody>
</table>
During the telephone follow-up one month post-trauma, six patients refused to continue participating in the study, and two additional cases were unreachable by telephone. Follow-up interviews were performed for 70 patients, all of whom completed the study. No patients had died in the month between injury and follow-up, and all patients had GCS scores of 12.

The mean serum S100B value was significantly lower for patients with minor TBI than for patients with moderate TBI (20.4 ± 12.6 ng/dl and 124.0 ± 235.0 ng/dl, respectively). Patients with normal CT scans also had statistically significantly lower serum S100B levels than patients with abnormal CT findings. The mean S100B value was statistically significantly higher for patients with suspected diffused axonal injury (596.18 ± 502.1 ng/dl) than for patients with other abnormal CT findings (p=0.000); 20.97 ± 19.9 ng/dl in patients with normal CT results; 39.56 ± 21.7 ng/dl in patients with skull bone fracture; 50.38 ± 22.9 ng/dl in patients with intracranial haemorrhage; and 70.23 ± 31.3 ng/dl in patients with fracture plus intracranial haemorrhage. Student’s t-test demonstrated that the difference was statistically significant (p=0.003). The mean S100B value was statistically significantly higher in patients with suspected DAI compared to patients with other abnormal CT findings (p=0.000). Serum S100B results are summarized in Table 2. Initial GCS scores, CT findings, headache, and Barthel scores of patients with Barthel scores <18 and with the highest S100B levels are shown in Table 2. Serum S100B levels were higher in patients with lower Barthel scores, but the difference was not statistically significant (p=0.06).

**DISCUSSION**

The S100B protein has a half-life of two hours and can be measured both in CSF and in the blood. Although some studies have shown that S100B protein levels increase after extra-cranial injuries in the absence of brain injury [23], many other studies have introduced S100B protein as a highly sensitive and specific biomarker of CNS injuries [20]. S100B has been suggested as a triage tool for identifying patients who need neuroimaging and as a diagnostic tool for early recognition of patients with possible brain tissue injury and timely administration of medication (e.g., benzodiazepines to reduce post concussion syndrome risk after mild TBI). S100B has also been suggested as a prognostic tool to identify at risk patients and to begin rehabilitation activities as soon as possible, especially for patients who do not need neurosurgical interventions [24] increases in minor to moderate traumatic brain injuries (especially in cases of DAI), it cannot accurately predict one-month outcomes. These results are compatible with some other studies which have emphasized the complicated release pattern of S100B. These past studies have highlighted the role of blood–brain barrier integrity and disruption in S100B release into the serum, the poor correlation between serum and CSF S100B levels, and the possible reparative roles of S100B that may improve outcomes in patients with acute brain injuries. These studies also mention that the relationship between S100B values and likely outcomes in patients with TBI are not necessarily a causative relationship [25]. A study of a large cohort of patients showed some association between high serum S100B level and poor outcome in patients with brain injury, but not significant enough to support use as an outcome prediction tool[26]. Similarly, a review by Town end showed that although patients with high serum S100B levels at initial evaluation may be at higher risk for disability after TBI, no association between serum S100B levels and the neuropsychological performance of injured patients has

<table>
<thead>
<tr>
<th>CT Findings</th>
<th>Mean ±SD (ng/dl)</th>
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<tbody>
<tr>
<td>Skull Fracture</td>
<td>39.56 ± 21.7</td>
</tr>
<tr>
<td>ICH</td>
<td>50.38 ± 22.9</td>
</tr>
<tr>
<td>Skull Fracture with ICH</td>
<td>70.23 ± 31.3</td>
</tr>
<tr>
<td>DAI</td>
<td>596.18 ± 502.1</td>
</tr>
<tr>
<td>Abnormal</td>
<td>124.0 ± 235.0</td>
</tr>
<tr>
<td>Normal</td>
<td>20.97 ± 19.9</td>
</tr>
</tbody>
</table>

Intracranial haemorrhage (ICH); diffused axonal injury (DAI); standard deviation (SD).
been established [27]. Metting et al. studied 94 patients with mild TBI and demonstrated that S100B is not related to outcome or imaging results [28]. Some newer studies have proposed that serum S100B level might be used for predicting the probability of brain death in patients with TBI [29].

**CONCLUSION**

The current study showed that serum S100B levels increase with minor to moderate TBIs, especially in patients with suspected DAI. However, serum S100B cannot accurately predict one-month neuropsychological outcomes and performance.

**REFERENCES**


Clinical Significance of Serum Biomarker S100B to Predict Outcome After Traumatic


