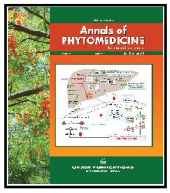
Annals of Phytomedicine 12(2): 645-654, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.2.75

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

S100B: A marker of severity in traumatic brain injury

Sriprajna Mayur and Usha Adiga◆

Department of Biochemistry, K S Hegde Medical Academy, Nitte (Deemed to be University), Mangalore-575018, Karnataka, India

Article Info

Article history

Received 4 November 2023

Revised 13 December 2023

Accepted 14 December 2023

Published Online 30 December 2023

Keywords

S100B

TBI

Glasgow coma score

Glasgow outcome score

Clinical outcome

Abstract

Exploring the potential of blood biomarkers and clinical indicators following traumatic brain injury (TBI) can offer valuable diagnostic and predictive insights. S100B, a well-studied blood biomarker in the context of TBI, has yielded inconclusive findings regarding its ability to distinguish TBI severity and predict outcomes. This study aims to verify the utility of S100B blood levels as predictive factors for TBI severity and patient outcomes. This study included 85 TBI patients (with Glasgow coma scale scores ranging from 3 to 15) admitted to the neurosurgery department. Blood serum samples were collected within 48 h of the traumatic incident and analyzed for S100B concentrations. Patient demographics, injury mechanisms, CT brain scan results, and Glasgow Coma scale scores were also recorded. Patient outcomes were evaluated using the Glasgow outcome scale extended, dichotomized into favourable vs. unfavourable and deceased vs. alive at three and six months post-injury. Upon admission, S100B serum levels significantly varied among mild, moderate, and severe TBI groups (p=0.000). Subsequent post hoc analysis confirmed a significant distinction in S100B concentration between mild vs. severe (p=0.000) and moderate vs. severe (p=0.004) TBI. S100B concentrations at admission effectively distinguished between favourable and unfavourable outcomes at three months of follow-up (p=0.000). Median serum levels were notably higher among deceased patients. The protein exhibited an inverse correlation with the initial Glasgow coma scale score and the Glasgow outcome scale extended outcome scores at three and six months, categorized as favourable (GOSE5-8) and unfavourable (GOSE1-4) outcomes. A positive correlation was observed with the total scores of Rotterdam CT brain categorization (p=0.289, p=0.005). S100B demonstrated moderate discriminative ability, with sensitivities of 72%, 71%, 72%, and 74% in predicting severity, mortality, and poor outcomes at three and six months, respectively. Incorporating on-admission S100B levels into the assessment of TBI may serve as an additional reliable tool for predicting patient outcomes and tailoring treatment strategies accordingly.

1. Introduction

A major health issue that is progressively affecting people worldwide is CNS diseases, particularly neurodegenerative disorders have gained focus in recent years,which have a high morbidity and mortality rate (Sharma *et al.,* 2021; Adnan *et al.,* 2022). In addition to mediating neurodegeneration, the damage to neurons causes deficiencies in spatial learning and memory in elderly people. (Arpitaa *et al.,* 2022; Sekeroglu *et al.,* 2019).India is currently undergoing significant socio-demographic and epidemiological changes due to rapid urbanization, industrialization, increased motorization, and shifts in lifestyle (Han *et al*., 2013). This transformation has given rise to various challenges, including the prevalence of Traumatic Brain Injury (TBI), which is a pressing concern in India. TBI contributes to both mortality and morbidity, particularly among the young and economically productive population (Puvanachandra *et al*., 2003). According to epidemiological data, India experiences approximately 1.6 million TBI cases each year. Shockingly, around 200,000 individuals succumb to brain injuries annually, and nearly 1 million people require access to rehabilitation programs (Jennett *et al*., 1998). Clinical characteristics of traumatic brain injury are studied in conjunction with diagnostic biomarkers and imaging tests (Ashtekar *et al*., 2023). Often referred to as the 'silent epidemic,' clinical recovery following a TBI can vary significantly, with some patients making remarkable progress while others struggle with significant disabilities (Gururaj *et al*., 2008). To address this critical issue, it becomes imperative to develop an effective prognostic model and treatment strategy based on available admission data. Extensive research efforts are currently underway in this domain, with a particular focus on exploring the utility of serum biomarkers to improve prognostic capabilities, thereby enhancing patient outcomes, aiding in drug discovery, and advancing therapeutic approaches.

One of the extensively studied proteomic markers in the context of TBI is the S100B calcium-binding protein. This protein is primarily found in white matter and is synthesized in astroglia and Schwann cells, where it plays a role in regulating intracellular calcium levels (Thornhill *et al*., 2000; Herrmann *et al*., 1999). When astrocytes experience injury or metabolic stress, they release accumulated S100B, which can be detected extracellularly in as little as 15 sec following an injury (Olsson *et al*., 2011). Moreover, S100B mRNA levels also increase shortly after injury, indicating ongoing protein synthesis within the cells (Willoughby *et al*., 2004). As a result, the detected S100B levels in the blood stem from both secreted and newly synthesized sources. Most of the S100B found in the serum is released from the cerebrospinal fluid (CSF) through arachnoid villi, establishing a correlation between the CSF:S100B ratio and the time elapsed after a TBI (Hinkle *et al*., 1997). Due to its rapid appearance in the serum

Corresponding author: Dr. Usha Adiga

Professor, Department of Biochemistry, KS Hegde Medical Academy, Nitte (Deemed to be University), Mangalore-575018, Karnataka, India

E-mail: ushachidu@yahoo.com

Tel.: +91-8277781638

post-injury, this protein has been included in Scandinavian guidelines for managing head injuries since 2013 (Goyal *et al*., 2013).

A notable advantage of using S100B in injury research is its stability and resilience to storage conditions and temperature fluctuations, making its handling convenient and ensuring reliable results, even after repeated freeze-thaw cycles (Thelin *et al*., 2017). It is also worth mentioning that S100B remains unaffected by hemolysis (Raabe *et al*., 2003).

The utility of S100B in discriminating injury severity and predicting outcomes has been a subject of extensive discussion over the years. However, conclusive evidence remains limited and sometimes conflicting, largely due to variations in the optimal time between trauma and sample collection. In this study, our objective was to analyze S100B levels in the serum upon hospital admission, evaluating its potential as a marker for assessing TBI severity and predicting outcomes, specifically in terms of Glasgow outcome scale extended (GOSE) scores at three and six months post-TBI.

This investigation aimed to explore the potential association between serum S100B levels, traumatic brain injury (TBI) severity, and subsequent clinical outcomes. The primary focus of this study was to shed light on the diagnostic and prognostic value of S100B protein as a biomarker in cases of TBI.

The significance of the S100B protein in this context lies in its release by glial cells in the brain. Elevated levels of S100B in the bloodstream may serve as an indicator of brain tissue damage. The researchers likely measured S100B levels in the blood of TBI patients and correlated these levels with clinical indicators of TBI severity, including Glasgow coma scale (GCS) scores, radiological findings, and clinical outcomes. The study's findings may reveal crucial insights, such as the potential correlation between higher S100B levels, more severe TBI, and poorer clinical outcomes. The use of S100B as a diagnostic and prognostic biomarker for TBI holds promise for clinical practice. However, the specific results and their implications will depend on the data collected, the statistical analyses conducted, and the characteristics of the study population. It's important to recognize that the use of serum S100B as a biomarker for TBI remains an active area of research, and the findings from this study will contribute to the existing body of knowledge in this field.

2. Materials and Methods

2.1 Methodology

2.1.1 Research design, ethical considerations, and study setting

This study was conducted within the framework of a prospective cohort investigation. The research participants consisted of individuals who had sustained head injuries and sought medical attention at the Neurosurgery department of Justice KS Hegde Charitable Hospital from December 2019 to September 2021. Ethical approval for this study was obtained from the Central Ethics Committee NITTE (Deemed to be University) under reference number NU/CEC/2019/0250. Consent for participation in the study was obtained from the next of kin, in accordance with the principles outlined in the Declaration of Helsinki.

2.1.2 Study population, sampling, and data collection

During the data collection period, a total of 111 patients were initially considered eligible for the study. However, due to the study's extended follow-up period of up to six months post-TBI, several participants were excluded for various reasons, including loss to follow-up (n=7), late admissions occurring more than 48 h after the injury (n=5), patients with uncontrolled systemic hypertension and diabetes (n=2), individuals with retro-positive reports (n=2), those testing positive for Covid-19 (n=1), cases involving spinal injuries (n=7), and patients diagnosed with cancer (n=2). Consequently, the final study cohort comprised 85 eligible patients (Figure 1).

Clinical and demographic information, as well as Glasgow coma scale (GCS) scores, were documented upon the patients' admission. Radiological findings obtained through computed tomography (CT) scans of the brain were categorized using the Rotterdam CT(R-CT) brain classification system (Table 1) (Beaudeux *et al*., 2000).

All head-injured patients enrolled in this study received treatment according to the established standard protocol at our tertiary care hospital.

Table 1: Description of Rotterdam CT (R-CT) brain score categories

CT brain features	Score
Basal cistern	
• Normal	0
• Compressed	1
• Absent	2
Midline shift	
• No shift or shift ≤ 5mm	0
• Shift >5mm	1
Epidural mass lesion	
• Present	0
• Absent	1
IVH o tSAH	
• Absent	0
• Present	1
Sum score	+1

IVH: Intraventricular haemorrhage;

tSAH: Traumatic subarachnoid haemorrhage.

2.1.3 Biomarker determination

2.1.3.1 Sample collection and preservation

Two milliliters of venous blood were aseptically collected from each patient using plain vacutainers within 48 h of the traumatic brain injury (TBI). The collected blood samples were subsequently subjected to centrifugation at 3000 revolutions per minute (rpm) for a duration of 10 min. The resulting serum was then carefully separated and preserved in a freezer set at a temperature of -30 degrees Celsius in preparation for further analysis.

2.1.3.2 S100B quantification

Quantitative analysis of serum S100B levels was conducted using the Enzyme linked immunosorbent assay (ELISA) method,

specifically employing a kit provided by XEMA Co., Ltd. This ELISA method allowed for the measurement of S100B within the detectable concentration range of 10 nanograms per liter (ng/l) to 3500 ng/l. It is important to note that a serum concentration of 90 ng/l represented the upper limit of detectable S100B concentration in healthy control samples.

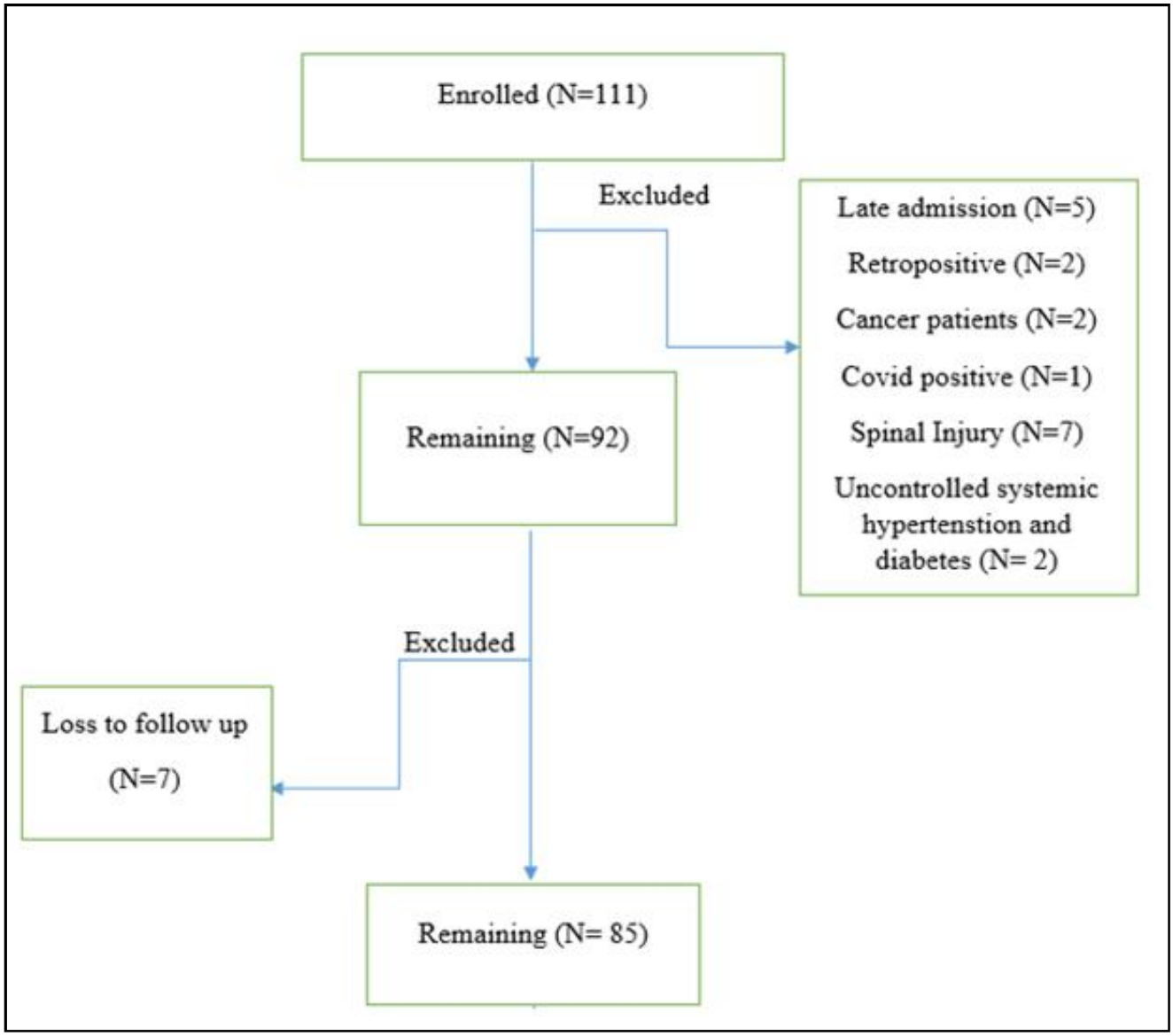


Figure 1: Flow chart depicting patients' enrolment for the study.

2.1.4 Outcome assessment

2.1.4.1 Clinical outcomes assessment

Clinical outcomes for the patients were assessed at two time points: three months and six months following the traumatic brain injury (TBI). This assessment was performed using the extended Glasgow outcome score (GOSE), which categorizes outcomes into eight distinct categories, as delineated in Table 2.

In accordance with the GOSE scoring system, a total score of 1 corresponds to a state of death, while a score of 2 represents a vegetative state. A score of 3 signifies severe disability, characterized by complete dependency on others, and a score of 4 indicates severe disability with partial dependency on caregivers.

Patients achieving a score of 5 exhibit independence but may be unable to resume work at their previous capacity. For those scoring 6, some degree of disability may persist, but they can partly resume their prior work or activities. A score of 7 indicates a good recovery with minor physical deficits that may affect daily life, while the highest score of 8 represents a complete and good recovery.

To facilitate analysis, the GOSE scores were further categorized into two groups: optimal outcomes (GOSE $\geq$ 5) and suboptimal outcomes (GOSE$\leq$4). This dichotomization allowed for a more straightforward evaluation of patient outcomes based on their GOSE scores.

Table 2: Description of categories of the Glasgow outcome scale extended

Score	Category	Domain	Criteria
1	Dead		
2	Vegetative state	Consciousness	Not able to look after themselves for 8 h.
3	Lower SD	Activities at home	Not able to look after themselves for a day
4	Upper SD	Activities outside home function	Not able to shop and travel
5	Lower MD	Ability to work or study social and leisure	Not able to work/studyNot able to participate
		activities friction in family and friendship	Constant frictions
6	Upper MD	Work social and leisure activities friction in family and friendship	Reduced work capacity less participation Frequent frictions
7	Lower GR	Social and leisure activities friction in family and friendship symptoms affecting daily life	Participate less occasional problemsYes
8	Upper GR	Symptoms affecting daily life	No problem

SD- Severe disability, MD- Moderate disability, GR- Good recovery

2.1.5 Statistical analysis

In this study, the statistical analysis was conducted using two software packages: the Statistical Package for the Social Sciences (SPSS) version 23.0 by IBM, Chicago, IL, US, and GraphPad Prism version 9.4.1.

Demographic and clinical features of the patients upon admission were summarized and presented as counts and percentages. To evaluate the distribution of numerical data, tests for normality were performed, including the Shapiro-Wilk and Kolmogorov-Smirnov tests.

S100B serum levels were compared among different groups based on TBI severity and patient outcomes. The Kruskal-Wallis test, followed by the Bonferroni post hoc test, was employed for comparisons between multiple groups, while the Mann-Whitney U test was used for two-group comparisons.

To assess the relationships between S100B serum levels and TBI severity, CT brain findings, and patient outcomes, Spearman's correlation analysis was applied.

The predictive value of S100B for injury severity, the extent of moderate to severe TBI according to the Glasgow coma scale (GCS) score, poor functional outcomes at three- and six-month follow-up, and mortality within six months of TBI were determined using receiver operator characteristics (ROC) analysis. An area under the curve (AUC) value in the range of 0.7 to 0.8 was considered indicative of acceptable discrimination.

Statistical significance was defined as a *p*-value less than 0.05, signifying that results were regarded as statistically significant.

3. Results

3.1 Participant characteristics and traumatic brain injury causes

Tables 3 and 4 provide an overview of the demographic and clinical attributes of the individuals who suffered head injuries and were included in the study. The study encompassed a wide age range, with participants spanning from 19 to 72 years, with a mean age of 39.7 ± 14.2. A majority of the individuals were male, constituting 76.5% of the total participants, and they exhibited diverse employment backgrounds.

The most prevalent cause of injury observed in this study was road traffic accidents (RTAs), accounting for 71.8% of the cases, followed by falls from height, which made up 22.4% of the injuries. A smaller proportion, 5.9%, resulted from other various causes.

The study's demographics and the factors contributing to traumatic brain injuries are presented in Tables 3 and 4. The participants' ages ranged from 19 to 72 years, with a mean age of approximately 39.7 years. A majority of the participants were male and engaged in various occupational activities. The leading cause of head injuries among the participants was road traffic accidents, followed by falls from heights and other, less common causes. This information provides a snapshot of the study population and the primary reasons behind traumatic brain injuries in the sample.

Table 3: Patient demographics

Variables	N (%)
Age	
≤ 30	28 (32.9)
31-50	41 (48.2)
Ã 50	16 (18.8)
Gender	
Male	65 (76.5)
Female	20 (23.5)
Employment status	
Agriculturist/Coolie	26 (30.6)
Employed/Business	34 (40)
Homemaker	12 (14.1)
Others	13 (15.3)
Mechanism of injury	
RTA	61 (71.8)
Fall from height	19 (22.4)
Other	5 (5.9)

The average systolic blood pressure was measured at 128.89 ± 20.8 mmHg, while the mean pulse rate was 83.7 ± 15.4. Unilateral pupillary responsiveness was noted in 28.2% of the individuals within the study population. A significant portion of the patients, constituting 73%, exhibited symptoms of loss of consciousness or posttraumatic amnesia (LOC/PTA). Furthermore, 18.8% of patients experienced seizures, 44.7% reported vomiting before hospitalization, and 20% and 33% of traumatic brain injury survivors presented with ear and nasal bleeding, as summarized in Table 4.

Table 4: Clinical features

Variables	Mean ± SD/N(%)
Systolic BP	128.89 ± 20.8
Pulse 83.7 ± 15.4	
Pupillary responsiveness	
Bilaterally responsive	61 (71.8)
Unilaterally responsive	24 (28.2)
LOC/PTA experienced	
Yes	62 (72.9)
No	23 (27.1)
Seizure	
Yes	16 (18.8)
No	69 (81.2)
Vomiting	
Yes	38 (44.7)
No	47 (55.3)
Ear bleed	
Yes	17 (20)
No	68 (80)
Nasal bleed	
Yes	28 (32.9)
No	57 (67.1)

Table 5 provides an overview of the CT brain findings, classified according to the R-CT brain categorization. Among the TBI subjects, 4.9% displayed partial compression of the basal cistern, and 2.4% exhibited complete compression. The presence of a midline shift was identified in 7.1% of the CT brain images. Epidural mass lesions were observed in a substantial 83.5% of the CT brain images. Additionally, 52.9% of head-injured patients were noted to have intraventricular hemorrhage (IVH) or traumatic subarachnoid hemorrhage (tSAH).

Table 5: CT brain findings under Rotterdam CT brain (RCT) categorisation

Variables	N (%)
Compression of basal cistern	
0	79(92.9)
1	4(4.9)
2	2(2.4)
Midline shift	
0	79(92.9)
1	6(7.1)
Epidural mass lesion	
0	71(83.5)
1	14(16.5)
IVH or tSAH	
0	40 (47.1)
1	45 (52.9)
RCT total score	
Abnormal (Ã1)	79 (92.9)
Normal (0 – 1)	6 (7.1)

The study analyzed S100B levels in relation to both the severity of injury and the outcomes. Based on the Glasgow coma scale (GCS) scores upon admission, the subjects were categorized into three groups: mild injury (39 subjects), moderate injury (25 subjects), and severe injury (21 subjects). Table 6 and Figure 2 present the median serum S100B concentrations, along with the interquartile range, within these three subgroups of injury severity. Among these subgroups, the lowest median concentrations were observed in the mild TBI subgroup. Notably, S100B concentrations increased progressively and significantly in the moderate and severe TBI subgroups (Kruskal-Wallis p=0.000). Post hoc (Bonferroni) testing further confirmed significant differences in S100B serum concentrations between the mild and severe TBI subgroups (p=0.000) and the moderate and severe TBI subgroups (p=0.004). This suggests a correlation between higher S100B levels and increased severity of traumatic brain injury.

Table 6: Comparison of S100B serum levels between severity groups

S100B ng/l	Mild TBI	Moderate TBI	Severe TBI	Kruskal Wallis p	Post-hoc (Bonferroni)	p value
N	39	25	21		Mild-moderate	1.0
Median	325.7	491.8	753.5	0.000*		
Lower IQR	261.32	317.4	412.8		Mild-severe	0.000*
Upper IQR	446.5	629.95	1073.1		Moderate-severe	0.004*

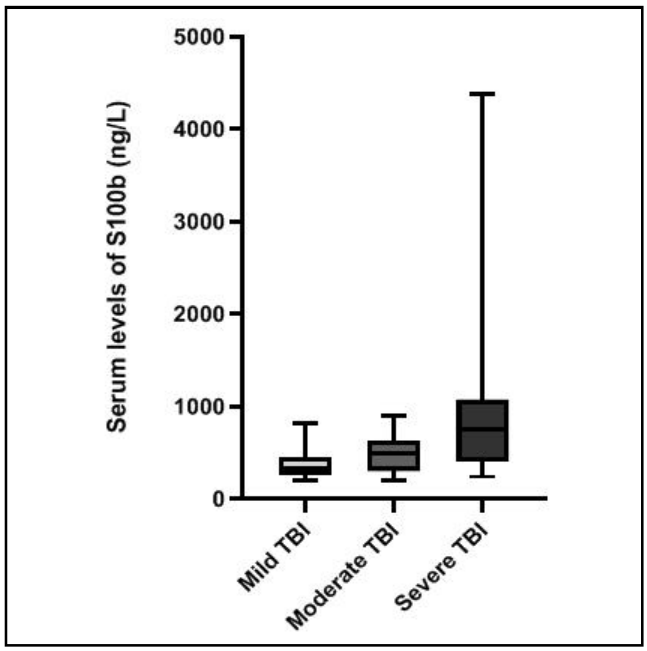


Figure 2: Illustrates boxplots displaying the concentrations of the biomarker (S100B) in subjects with mild, moderate, and severe traumatic brain injury (TBI). These boxplots provide a visual representation of the distribution of S100B values within each group.

In each boxplot:

- The horizontal line inside the box represents the median serum S100B value.
- The box itself spans the interquartile range, with the lower and upper boundaries marking the first and third quartiles of the data.
- The whiskers extend from the box to the lowest and highest observed values within a specific range below the upper fence.

The first box corresponds to subjects with mild TBI, including 39 individuals. The middle box represents those with moderate TBI, totaling 26 subjects, and the severe TBI group consists of 21 subjects. These boxplots effectively illustrate the variability and central tendency of S100B concentrations across different levels of TBI severity.

The receiver operating characteristic (ROC) analysis provided further support for S100B's capability to evaluate TBI severity. It identified a threshold value of ≥378 ng/l (Figure 3) with a sensitivity of 72% and a specificity of 70% (1-0.308). The Area under the curve (AUC) was calculated at 0.727, indicating a statistically significant and robust discriminative ability (p=0.000).

The ROC analysis is a statistical tool used to assess the performance of a diagnostic test or biomarker, such as S100B in this case. The analysis generates a curve (ROC curve) that visually represents the trade-off between sensitivity (the ability to correctly identify true positives) and specificity (the ability to correctly identify true negatives) across various threshold values. The AUC summarizes this trade-off, with a value closer to 1 indicating a better diagnostic performance.

In this specific analysis, an S100B level of ≥378 ng/l was identified as the optimal threshold for assessing the severity of TBI. When this threshold was used, the biomarker demonstrated a sensitivity of 72%, which means it correctly identified 72% of individuals with severe TBI. The specificity was 70%, indicating that it correctly identified 70% of individuals without severe TBI. The AUC of 0.727, which is significantly greater than 0.5, implies that S100B has a strong ability to distinguish between different TBI severities, and this result is statistically significant (p=0.000).

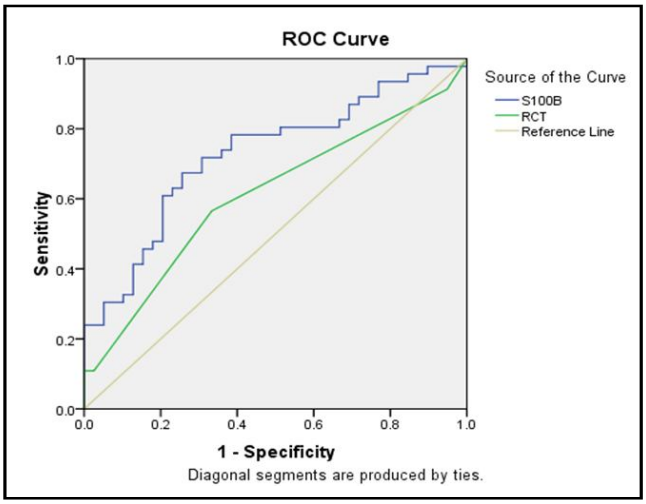


Figure 3: Receiver operating characteristic (ROC) curve for severity prediction in TBI using S100B.

During the follow-up assessments, unfavourable outcomes were observed in 32 and 23 individuals, while 53 and 62 individuals showed favourable outcomes at three and six months, based on their GOSE scores (Table 7). The median serum S100B levels upon admission were significantly higher among those who experienced unfavourable outcomes during the third-month follow-up (p=0.000). However, there was no statistically significant difference in S100B levels between outcome groups at the sixth-month assessment (p=0.63). The Area Under the ROC Curves (AUC) for predicting poor functional outcomes at three and six months was 0.729, with a threshold value of ≥ 406 ng/l, a sensitivity of 72%, specificity of 68%, and a statistically significant result (p=0.000) (Figure 4a). The AUC for the sixth-month assessment was 0.731, with a threshold value of ≥ 406 ng/l, a sensitivity of 74%, specificity of 63%, and a statistically significant result (p=0.001) (Figure 4b).

Out of the 14 patients who did not survive, there was a significantly higher concentration of S100B in their blood upon admission compared to the survivors (p=0.012), as described in Table 8. Patients with TBI who experienced mortality within six months of injury had elevated S100B levels, with a threshold value of ≥ 487.7 ng/l (Figure 5). The AUC was 0.714, with a sensitivity of 71%, specificity of 65%, and a statistically significant result (p=0.012).

The overall scores of the Rotterdam CT (R-CT) brain findings did not provide a significant threshold value, except for the three-month follow-up assessment, where the AUC was 0.673 with a threshold value of ≥2.5. This threshold had a sensitivity of 65%, specificity of 66%, and a statistically significant result (p=0.008) in predicting the likelihood of a poor outcome for patients with a total R-CT score exceeding 2.5 in their on-admission brain imaging (Figure 4a).

Table 7: Comparison of S100B serum levels between outcome groups during 3- and 6-month follow-up

S100B ng/l	Unfavourable outcome GOSE (1-4)	Favourable outcome GOSE (5-8)	Mann-whitney U *p*
Three months outcome			
N	32	53	
Median	570.18	334.98	0.000*
Lower IQR	370.9	264.9	
Upper IQR	945.57	483.7	
Six months outcome			
N	23	62	
Median	612.3	342.5	0.63
Lower IQR	399.75	275.16	
Upper IQR	1073.1	275.16	

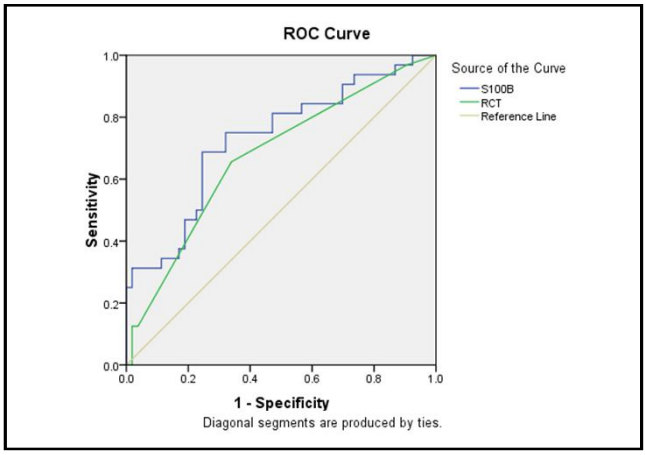


Figure 4a: Receiver operating characteristic (ROC) curve for outcome prediction in TBI with S100B levels measured <48 h and Glasgow outcome score extended (GOSE) for poor outcome scores at three months.

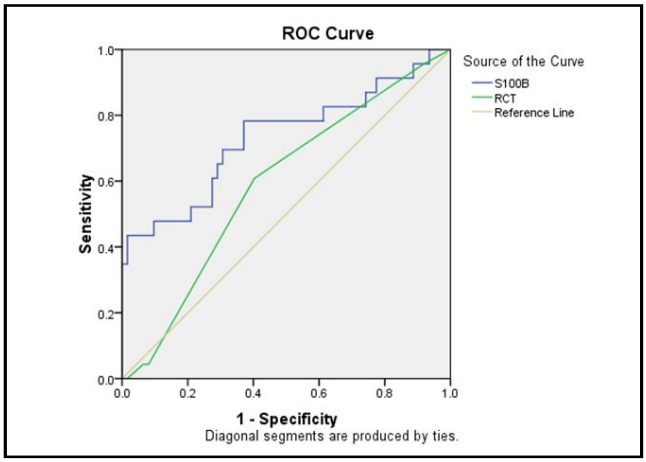


Figure 4b: Receiver operating characteristic (ROC) curve for outcome prediction in TBI with S100B levels measured <48 h and Glasgow outcome score extended (GOSE) for poor outcome scores at six months.

Table 8: Comparison of S100B serum concentration between non-survivors and survivors six months post-TBI

S100B ng/l	Non-survivors GOSE (1)	Survivors GOSE (2-8)	Mann-whitney U *p*
N	14	71	
Median	662.35	348.02	0.012*
Lower IQR	399.75	280.53	
Upper IQR	1123.8	595.1	

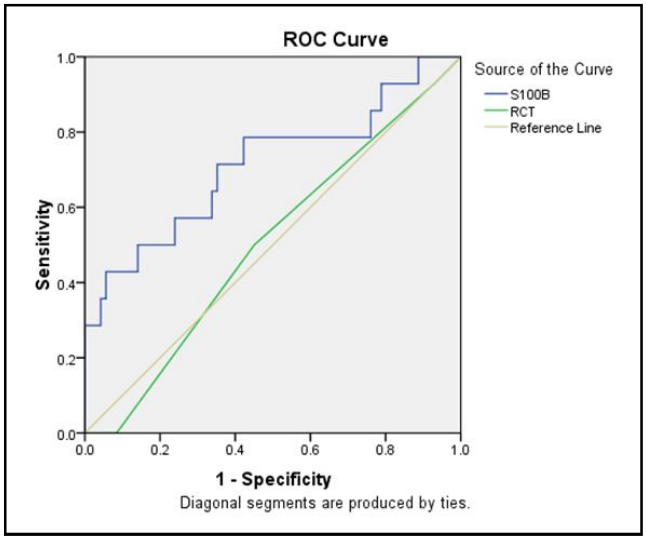


Figure 5: Receiver operating characteristic (ROC) curve for mortality prediction in TBI using S100B.

Upon analyzing the serum S100B levels in the entire study population, we observed several correlations, as outlined in Table 9. Notably, there was a significant negative correlation between S100B levels and the Glasgow Coma Scale (GCS) score upon admission (p=–0.3991, p=0.0002), as well as with the three-month and six-month Glasgow outcome scale extended (GOS E) outcome scores (p=–0.436, p<0.0001 and p=–0.388, p=0.0002, respectively).

Table 9: Correlation of S100B serum levels on admission with injury severity, CT brain (Rotterdam scores) and outcomes

Variables	Spearman's *p*	*p* value
Glasgow coma score (admission)	-0.3991	0.0002*
Rotterdam CT brain score		
Compression of basal cistern	0.1378	0.20
Midline shift	0.1385	0.20
Epidural mass lesion	0.05688	0.60
IVH or tSAH	0.3138	0.003*
Total score	0.2894	0.005*
Glasgow outcome score extended		
Three months follow-up	-0.436	<0.0001*
Six months follow-up	-0.388	0.0002*

S100B levels exhibited a significant positive correlation with the Rotterdam CT(R-CT) total score upon admission (p=0.289, p=0.005). However, the S100B levels did not display statistically significant correlations with different groups categorized based on radiological features such as basal cistern compression, midline shift, and epidural mass lesions, except for the group with intraventricular hemorrhage (IVH) or traumatic subarachnoid hemorrhage (tSAH), which showed a positive correlation (p=0.313, p=0.003).

4. Discussion

Differences in S100B levels across the severity groups: Our analysis of serum S100B concentrations in our study population indicated the capability of stratifying patients based on their injury severity, as determined by Glasgow Coma scale (GCS) scores (Kruskal-Wallis p=0.000) (Table 6). This finding aligns with several previous studies that utilized S100B within 6 to 24 h of injury to stratify patients by severity (Thelin *et al*., 2013; Koivikko *et al*., 2022; Herrmann *et al.,* 2000; Romner *et al*., 2001). Our study, however, exhibited a significant difference among all three severity groups. Further confirmation via the Bonferroni post hoc test underscored S100B's ability to distinguish between mild and severe and moderate and severe TBI groups (Table 6).

Correlation of S100B with GCS scores:We identified a significant negative correlation between S100B levels and the GCS score upon hospital admission. This correlation suggests that S100B can serve as a valuable supplementary marker for assessing injury severity (Table 9). A study reported a similar negative correlation between GCS and S100B collected within 48 h of TBI, consistent with our findings (Shakeri *et al*., 2013). Several studies that implemented serial sampling procedures have also observed significant negative correlations between the GCS score upon hospital admission and serum S100B collected on various days following TBI (Abdelfattah *et al*., 2020).

Comparison of S100B on admission with outcome groups:Our study demonstrated comparable results at three months of follow-up, with a Mann-whitney U p-value of 0.000. It is noteworthy that not many studies have reported differences in on-admission serum S100B levels between poor and good outcome groups. However, Hellwell *et al.* (2020) observed higher day one serum S100B levels in the moderate to severe TBI cohort with poor outcomes (GOSE 1-4) compared to those with good outcomes (GOSE 5-8), with a p-value of 0.008, consistent with our findings.

Correlation with CT brain findings: Our study population exhibited a significant positive correlation with CT brain findings categorized under Rotterdam CT scores (Table 9), with Spearman p=0.2894, p=0.005. Similar findings were recently reported in a study of the Chinese population by Yin *et al.* (2021). Additionally, Thelin *et al.* (2014) found a correlation between the radiographic image and the second peak of S100B at 48 h post-injury.

Correlation with the outcome: In our outcome measurements, we treated the population as continuous data and uniformly dichotomized GOSE scores into good (GOSE 5-8) and poor (GOSE 1-4) outcomes. evertheless, many studies have used outcome measurements after grouping their study populations by GCS scores, as discussed below.

Our study identified a negative correlation between S100B serum levels upon admission and GOSE outcomes at three and six months. Townend *et al.* (2008) also found a similar correlation at the one-month follow-up in a study with a population comprising all three severity categories. Furthermore, in the study involving severe TBI subjects and serial sample collection, 48-h serum S100B negatively correlated with three-month GOSE scores. Another cohort of severe TBI subjects with serial sampling over five days exhibited a significant negative correlation between serum S100B collected at the three-month follow-up and initial, 72-h, maximum release, and total release of S100B (Walder *et al*., 2013). In contrast, Metting, *et al*. (2012) included only mild TBI patients in their study, dichotomizing GOSE as optimal=8 and suboptimal<8, and reported no correlation between the biomarker released upon admission and six-month outcomes.

The predictive ability: We discovered a moderate discriminative ability (AUC= 0.714, sensitivity 71%, specificity 65%, and p=0.012) for serum S100B levels between non-survivors and survivors of TBI, as evidenced by Mann-Whitney p=0.012, with serum levels being significantly higher in non-survivors. This finding is consistent with Rodríguez-Rodrigueza *et al*. (2016) published results. They explained that serum samples collected within 48 hours of injury were a better predictor of mortality, with higher levels of S100B among non-surviving participants. Notably, our study's notable finding was the ability of S100B to predict mortality within 48 hours of injury in a population of mixed severity.

It is important to note that earlier studies have highlighted the short half-life of S100B, with levels returning to baseline within 7 h, following a brain injury, limiting the window for serum collection (Ingebrigtsen *et al*., 1999). However, the majority of patients in our tertiary care setup were referred to us beyond 24 h after TBI, following primary care at local nursing homes or primary health centers. Financial constraints also contributed to this delay. In a review addressing the distribution of neurologists and neurosurgeons in India, Ganapathy *et al*. (2013) mentioned that approximately 700 million rural Indian residents must travel more than 75-100 km for tertiary consultations. This scenario is common in the majority of our study subjects, making it practically challenging to reach a tertiary care hospital within a brief timeframe after injury. Therefore, serum collection within the first 48 h may hold promise as a diagnostic and prognostic tool for injury severity and outcome, especially for less privileged rural patients who face difficulties in seeking prompt medical care. Alongside other established prognostic tools, a single S100B biomarker level measurement within 48 h may contribute to personalized care and medication strategies for TBI patients.

5. Conclusion

In conclusion, the correlation between S100B levels and Rotterdam CT Brain classification scores has the potential to enhance prognostic accuracy and assist in determining the severity of intracranial injuries. The bulk release of serum S100B in the first 48 h, following TBI appears to be the most accurate timing for diagnosing injury severity at the time of admission and predicting death and favourable or unfavourable outcomes at three and six months following the TBI.

We acknowledge several limitations of this study.

1. We did not conduct a statistical analysis of pupillary responsiveness data in relation to serum S100B levels and their predictive value for TBI outcomes.

2. The speed of recovery varies for each TBI case, and in this study, we did not dichotomize the population according to the severity group (GCS) and divide it into Mild and Moderate + Severe TBI. Applying the GOSE score for 3- and 6-month follow-ups based on the injury severity would have provided greater insights into S100B's predictive ability.

Future goals

Serum biomarkers of head injury have not been extensively explored in India. In this study, we only considered GOSE scores to assess the functional outcomes of head-injured subjects, which primarily address physical well-being. Individuals who have suffered TBI also encounter significant challenges related to their emotional and social aspects of life, particularly in the Indian (rural) context, which remains less discussed. In our future research, we aim to investigate the ability of serum biomarkers to predict neurocognitive functioning, emotional trauma, and other psychosocial aspects experienced by TBI patients.

Acknowledgements

Dr Sucheta Kumari, Incharge of Central Research Laboratory for her support to utilize laboratory. Received the grants from Research society for the study of diabetes of India.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Abdelfattah, M. E.; Nafie, M.; Abdelwahab, K. and Fahmy, A. (2020). Prognostic value of protein S100B in traumatic brain injury. Egyptian Journal of Critical Care Medicine, **7**(2):80-85.

Ashtekar, H,; Varghese, N,; Anigol, P,; Ameen, M. M,; Dsouza, S,; Dsouza, S.S, and Balkur S. (2023). A review on recent pharmacological screening models to evaluate Parkinson's. Ann. Phytomed., **12**(1):31-38.

Beaudeux, J. L.; Leger, P.; Dequen, L.; Gandjbakhch, I.; Coriat, P. and Foglietti, M. J. (2000). Influence of hemolysis on the measurement of S-100â protein and neuron-specific enolase plasma concentrations during coronary artery bypass grafting. Clinical Chemistry, **46**(7):989-990.

Ganapathy, K. (2013). Neurosurgery in India: An overview. World Neurosurgery, **79**(5-6), 621-628.

Goyal, A.; Failla, M. D.; Niyonkuru, C.; Amin, K.; Fabio, A.; Berger, R. P. and Wagner, A. K. (2013). S100b as a prognostic biomarker in outcome prediction for patients with severe traumatic brain injury. Journal of Neurotrauma, **30**(11):946-957.

Gururaj, G. (2002). Epidemiology of traumatic brain injuries: Indian scenario. Neurology Research, **24**(1):24-28.

Han, G.; Wang, O.; Nie, M.; Zhu, Y.; Meng, X.; Hu, Y.; Liu, H. and Xing, X. (2013). Clinical phenotypes of Chinese primary hyperparathyroidism patients are associated with the calcium-sensing receptor gene R990G polymorphism. European Journal of Endocrinology, **169**(5): 629-638.

Hellewell, S. C.; Conquest, A.; Little, L.; Vallance, S.; Board, J.; Bellomo, R. and Morganti-Kossmann, M. C. (2020). EPO treatment does not alter acute serum profiles of GFAP and S100B after TBI: A brief report on the Australian EPO-TBI clinical trial. Journal of Clinical Neuroscience, **76**:5-8.

Herrmann, M.; Curio, N.; Jost, S.; Wunderlich, M. T.; Synowitz, H. and Wallesch, C. W. (1999). Protein S-100B and neuron-specific enolase as early neurobiochemical markers of the severity of traumatic brain injury. Restorative Neurology and Neuroscience, **14**(2-3):109-114.

Herrmann, M.; Jost, S.; Kutz, S.; Ebert, A.; Kratz, T.; Wunderlich, M. T. and Synowitz, H. (2000). Temporal profile of release of neurobiochemical markers of brain damage after traumatic brain injury is associated with intracranial pathology as demonstrated in cranial computerized tomography. Journal of Neurotrauma, **17**(2):113-122.

Hinkle, D. A.; Baldwin, S. A.; Scheff, S. W. and Wise, P. M. (1997). GFAP and S100â expression in the cortex and hippocampus in response to mild cortical contusion. Journal of Neurotrauma, **14**(10):729-738.

Ingebrigtsen, T.; Waterloo, K.; Jacobsen, E. A.; Langbakk, B. and Romner, B. (1999). Traumatic brain damage in minor head injury: Relation of serum S-100 protein measurements to magnetic resonance imaging and neurobehavioral outcome. Neurosurgery, **45**(3):468-475.

Injamuri, A.; Chellammal, H. S. J.; Diwan, P. V. and Yathavakilla, S. (2022). Neuroprotection of *Prunus avium* Linn. extract on cerebral ischemic stroke in rats associated with neuroendocrine effects. Ann. Phytomed., **11**(1);283-288.

Jennett, B. (1998). Epidemiology of head injury. Archives of Disease in Childhood, **78**(5):403-406.

Koivikko, P.; Posti, J. P.; Mohammadian, M.; Lagerstedt, L.; Azurmendi, L.; Hossain, I. and Thelin, E. P.; Nelson, D. W. and Bellander, B. M. (2017). A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. Acta Neurochirurgica, **159**:209-225.

Maanpää, H. R. (2022). Potential of heart fatty-acid binding protein, neurofilament light, interleukin-10 and S100 calcium-binding protein B in the acute diagnostics and severity assessment of traumatic brain injury. Emergency Medicine Journal, **39**(3):206-212

Metting, Z.; Wilczak, N.; Rodiger, L. A.; Schaaf, J. M. and Van Der Naalt, J. (2012). GFAP and S100B in the acute phase of mild traumatic brain injury. Neurology, **78**(18):1428-1433.

Olsson, B.; Zetterberg, H.; Hampel, H. and Blennow, K. (2011). Biomarker-based dissection of neurodegenerative diseases. Progress in Neurobiology, 95(4):520-534.

Puvanachandra, P. and Hyder, A. A. (2009). The burden of traumatic brain injury in Asia: A call for research. Pakistan Journal of Neurological Sciences, **4**(1):27-32.

Raabe, A.; Kopetsch, O.; Gross, U.; Zimmermann, M. and Gebhart, P. (2003). Measurements of serum S-100B protein: effects of storage time and temperature on pre-analytical stability. Clinical Chemistry and Laboratory Medicine, **41**(5):700-703.

Rodríguez-Rodríguez, A.; Egea-Guerrero, J. J.; Gordillo-Escobar, E.; Enamorado-Enamorado, J.; Hernández-García, C.; Ruiz de Azua-Lopez, Z. and Murillo-Cabezas, F. (2016). S100B and neuron-specific enolase as mortality predictors in patients with severe traumatic brain injury. Neurology Research, **38**(2):130-137.

Romner, B.; Ingebrigtsen, T.; Kongstad, P. and Børgesen, S. E. (2000). Traumatic brain damage: serum S-100 protein measurements related to neuroradiological findings. Journal of Neurotrauma, **17**(8):641-647.

Sekeroglu, N. and Gezici, S. (2019). *Astragalus neurocarpus* Bioss. as a potential source of natural enzyme inhibitor associated with Alzheimer's and Parkinson diseases along with its rich polyphenolic content and antioxidant activities. Ann. Phytomed., **8**(1):82-87.

Sharma, S.; Kochhar, K.P.; Jain, S.; Jayasundar, R. and Divya, M.R. (2021) Therapeutic potential of Indian traditional medicines in Parkinson's disease. Ann. Phytomed., **10**(2):252-63.

Shakeri, M.; Mahdkhah, A. and Panahi, F. (2013). Posttraumatic brain death predictor biomarkers; S100B protein levels in association with patients' outcome. Archives of Trauma Research, **2**:76-80.

Thelin, E. P.; Johannesson, L.; Nelson, D. and Bellander, B. M. (2013). S100B is an important outcome predictor in traumatic brain injury. Journal of Neurotrauma, **30**(7):519-528.

Thelin, E. P.; Nelson, D. W. and Bellander, B. M. (2014). Secondary peaks of S100B in serum relate to subsequent radiological pathology in traumatic brain injury. Neurocritical Care, **20**(2):217-229.

Thornhill, S.; Teasdale, G. M.; Murray, G. D.; McEwen, J.; Roy, C. W. and Penny, K. I. (2000). Disability in young people and adults one year after head injury: prospective cohort study. BMJ., 320(7250):1631-1635.

Townend, W. J.; Guy, M. J.; Pani, M. A.; Martin, B. and Yates, D. W. (2002). Head injury outcome prediction in the emergency department: A role for protein S-100B? Journal of Neurology, Neurosurgery and Psychiatry, **73**(5):542-546.

Walder, B.; Robin, X.; Rebetez, M. M.; Copin, J. C.; Gasche, Y.; Sanchez, J. C. and Turck, N. (2013). The prognostic significance of the serum biomarker heart-fatty acidic binding protein in comparison with s100b in severe traumatic brain injury. Journal of Neurotrauma, **30**(19):1631-1637.

Willoughby, K. A.; Kleindienst, A.; Müller, C.; Chen, T.; Muir, J. K. and Ellis, E. F. (2004). S100B protein is released by in vitro trauma and reduces delayed neuronal injury. Journal of Neurochemistry, **91**(6):1284-1291.

Yin, W.; Weng, S.; Lai, S. and Nie, H. (2021). GCS score combined with CT score and serum S100B protein level Can evaluate severity and early prognosis of acute traumatic brain injury. Nan Fang Yi Ke Da Xue Xue Bao, **41**(4):543-548.

Citation **Sriprajna Mayur and Usha Adiga (2023). S100B: A marker of severity in traumatic brain injury. Ann. Phytomed., 12(2):645-654. http://dx.doi.org/10.54085/ap.2023.12.2.75.**