EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 6
66-74
Year: 2020

S-100 CALCIUM BINDING PROTEIN AS PROGNOSTIC FACTOR IN PATIENTS WITH TRAUMATIC BRAIN INJURY

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Article Received on 01/04/2020 **Article Revised on 22/04/2020** **Article Accepted on 12/05/2020**

ABSTRACT

Background: Traumatic brain injury (TBI) is the major cause of disability, morbidity, and mortality among individuals younger than 45 years. Age is a strong prognostic factor following TBI compared to other well-accepted prognostic variables such as Glasgow Coma Scale (GCS) and computed tomography category, S-100 B protein is another prognostic factor following TBI and it is the dominant biomarker of TBI in many studies. **Materials and methods:** This is a cross-sectional hospitalized-based study conducted in the National Center for Neurological Sciences (NCNS), during the period from October 2015 to October 2018**.** All patients clinically and radiologically diagnosed with TBI at the NCNS during the aforementioned period was enrolled in the study. Patients with non-traumatic brain injury were excluded from this study. Venous blood samples were collected from all participants in sterile plain containers for S-100 protein measurement by Enzyme-Linked Immunosorbent Assay. CT image findings of each patient were recorded and the outcome of patients was determined using the Glasgow Outcome Score (GOS).**Results:** Eighty-nine patients with TBI were enrolled in the study, 85.4% were males and 14.6% were female. The most affected age group ranged between 19-34 years in 40 patients. The results of this study indicated that 97.7% of the patients showed S-100 protein > 100 ng/L (Reference value=<100 ng/L) P=0.000. **Conclusion:** The findings of this study showed an association between age and outcome after TBI and an association between S-100 B protein level>150 ng/L and outcome after TBI but not significant to use this marker as a prognostic marker.

KEYWORDS: Traumatic brain injury, S-100 B protein, prognostic factor, outcome, Sudanese patients.

INTRODUCTION

Traumatic brain injury (TBI) is the major cause of disability, morbidity, and mortality among individuals younger than 45 years and is responsible for a significant proportion of all traumatic deaths in the United States and other developed nations.[1] TBI injury is defined as an alteration in brain function that manifests as altered level of consciousness, seizure, coma, or focal sensory or motor neurologic deficit resulting from blunt or penetrating force to the head.[2] Intracranial bleeding (IB) is a common and serious consequence of traumatic brain injury.[3] It may be due to physical trauma or nontraumatic causes such as a ruptured aneurysm.[4] Intracranial bleeding can be classified according to anatomical location, into epidural hemorrhage (EDH) subdural hemorrhage (SDH) intraparenchymal hemorrhage (IPH) and subarachnoid hemorrhage (SAH).[3]

Prognosis after TBI remains a challenge. Putative predictors are an active area of TBI research at present, and these studies are challenging long-held concepts in the field in addition to identifying possible new therapeutic strategies.[5] For instance, some studies suggest that a patient's sex may influence the outcome. Several factors are recognized modifiers of outcome from TBI.[5]

Age is a strong prognostic factor following TBI compared to other well-accepted prognostic variables such as Glasgow Coma Scale (GCS) and computed tomography category.[6] Advancing age is strongly associated with worsened prognosis, and an age

threshold was once proposed[7]; however, current evidence suggests a continuous relationship between increasing age and worsened outcome following TBI.[8] Also, TBI is the most common cause of death and disability in the pediatric population and this group associated with the poorer outcome despite the plasticity inherent in the immature brain.[9]

S-100 B protein is another prognostic factor following TBI and it is the dominant biomarker of TBI in many studies.[10] S100B is synthesized by astrocytes, oligodendrocytes and Schwann cells and represents about 0.2% of the total brain proteins. Brain injury causes a selective leakage of S100B into the cerebrospinal fluid and then into the blood, where measurements of S100B levels were found to be good indicators for the assessment of patients with cerebral ischemia due to stroke.[11] Similarly, blood levels of S100B can be used to monitor malignant melanomas and pediatric patients undergoing corrective cardiac surgery.[11] This protein belongs to a family of intracellular calcium-binding proteins predominantly present in mature perivascular astrocytes, neural progenitor cells and certain neuronal populations[12,13] In resting cells, S100 proteins are localized in specific cellular compartments from which some of them relocate upon cellular stimulation and even are secreted extracellular as cytokine-like activity, this might suggest that translocation might be a temporal or spatial determinant of their interactions with different partner proteins. S100B, for example, interacts with other A lzheimer's disease-associated proteins such as presenilin and with the amyloid precursor protein (APP). These interactions are altered by the phosphorylation state often and the overexpression of S100B; strongly indicating that S100B plays an important role in pathways associated with neurodegeneration.[11] Several studies analyzed how S100B may be utilized in moderate and severe TBI patients.[14,15,16,17] As severely brain-injured patients enter the emergency department and the intensive care unit, they are often sedated and/or intubated making neurological assessments problematic. While CT examinations aid in the diagnosis and surgical planning, it is often difficult to fully predict the outcome of these patients.[10] The capabilities of S100B to predict outcome have led some authors to conclude that S100B could be used as a marker of brain death following injury.[18,19] S100B appears to be an important and useful predictor of functional outcome in moderate-to-severe TBI.[10] This study aims to estimate the S-100 B proteins in serum and correlate the levels with computed tomography (CT) findings and to evaluate the influence of different age thresholds and S-100 B proteins on outcome following TBI.

MATERIAL AND METHODS

This is a cross-sectional hospital-based study conducted in the National Center for Neurological Sciences (NCNS), during the period from October 2015 to October 2018. All patients clinically and radiologically diagnosed with TBI at the NCNS during the aforementioned period were enrolled in the study. Patients with non-traumatic brain injury were excluded from this study. A pre-designed structural Questionnaire was used for the demographic and clinical data. Venous blood samples were collected from all participants in sterile plain containers for S-100 protein measurement. CT image findings of each patient were recorded and the outcome of patients was determined using the Glasgow Outcome Score (GOS). Verbal and written consent from each participant was obtained. Ethical clearance was obtained from the Ethical Review Board of NCNS.

Measurement of S100 Protein by Enzyme-Linked Immunosorbent Assay

The human S-100 antigen is combined with purified human S-100 antibody and labeled HRP to form antibody –antigen-enzyme-antibody complex which reacts with MTB substrate solution producing blue color at HRP enzyme-catalyzed, this reaction is terminated by adding stop solution and the color change is measured at a wavelength of 450 nm. The diluted standard was prepared by adding 50 µL of standard dilution in each tube, and then about 100µL of standard with concentration 2250ng/L was added to the first tube, after that 100 µL was transferred from the first tube into the second one. From the second to the fifth tube serial dilutions of the standard were obtained. The samples were then diluted by adding 10 µL of the sample to 40 µL of diluents' solution in sample well and mixed gently then incubated for 30 minutes at 37˚C. The diluted washing solution was added to every well, stilled for 30 seconds then drained; this step was repeated five times. After that, the enzyme (HRP-conjugate reagent) was added to each well except blank well and incubated for 30 minutes at 37˚c. A microtiter plate was then washed, and 50 µL of chromogen solution A was added to each well followed by the addition of 50 µL of chromogen solution B also to each well. Finally, the reaction was stopped by adding 50 µL of stop solution to each well. After 15 minutes, the optical density was obtained at 450 nm by ELISA reader and the concentration of S-100 protein was determined by comparison of the optical density of the samples to the standard curve.

RESULTS

Eighty-nine patients with TBI were enrolled in the study, 85.4% were males and 14.6% were females. The most affected age group ranged between 19-34 years in 40 patients. (Table1). Mild TBI was found in 60 % followed by moderate TBI in 28.8 % and severe TBI in 11.2% (Table2). Ninety percent of the patients had no association with any history of medical illness. Hypertension was encountered in 5.6 % of the patients and diabetes in 2.2% (Table3). Subdural hematoma was found in 20.8%, followed by Hemorrhagic contusion in18.5% of the cases and extradural hematoma in 14.9%. Results of other types of injury were displayed in (Table4). Finally, patient's outcomes according to the

Glasgow Outcome Score (GOS) is displayed in Table 5, mild disability being reported in 58.4% of the patients.

Table 1: shows age in the group of TBI.

		Frequency	Percentage %	P-value
Age in groups/Years	3 to 18	9	10.1%	**.000**
	19 to 34	40	45%	
	35 to 50	11	12.4%	
	51 to 66	18	20.2%	
	67 to 82	9	10.1%	
	83 to 98	2	2.2%	
Total		89	100%	

Table 2: shows the frequency distribution of GCS in TBI patients.

		Frequency	Percentage %
GCS	Severe	10	11.2%
	Moderate	23	25.8%
	Mild	56	63.0%
Total		89	100%

Table 3: shows the frequency distribution of history of past medical illness in TBI patients.

		Frequency	Percentage %
History of past medical illness	No history of past medical illness	80	90.0%
	Hypertension	5	5.6%
	diabetes	2	2.2%
	Heart disease	1	1.1%
	Asthma +diabetes	1	1.1%
Total		89	100%

Table 4: shows computed tomography findings in TBI patients.

		Frequency	Percentage %
computed tomography findings	Brain edema	6	6.9%
	Hemorrhagic Contusion	16	18.5%
	axonal injury	6	6.9%
	Extradural hematoma	13	14.9%
	Subdural hematoma	20	20.8%
	Edema + contusion	4	4.7%
	Bullet injury	1	1.1%
	SDH+contusion	3	3.5%
	SAH	6	6.9%
	EDH+SDH+HC	4	4.6%
	SAH +edema	1	1.1%
	HC+SAH	2	2.3%
	EDH+contusion	2	2.3%
	normal	1	1.1%
	H.C+SDH+Edema	1	1.1%
	Epidural Hemorrhage	1	1.1%
	axonal injury +Edema	1	1.1%
	Edema+SAH+HC	1	1.1%
Total		89	100%

Table 5: shows the frequency distribution of outcome according to Glasgow Outcome Score in TBI patients.

	GOS	Clinical status	Frequency	Percentage%
Outcome	1	Death	12	13.5%
	2	Vegetative	5	5.6%
	3	Severe disability	9	10.1%
	4	Moderate disability	11	12.4%
	5	Mild disability	52	58.4%
Total			89	100%

Regarding the age group, there was a statistical significant association between Age group and GOS P= 0.029 (Table 6). The results of this study indicated that 97.7% of the patients showed S-100 protein > 100 ng/L (Reference value=<100 ng/L). P=0.000 (Figure 1). The correlation between S-100 B protein and CT findings, GCS, and GOS were displayed in tables (6, 7, 8).

Table 6: shows cross tabulation between age group and outcome of TBI.

		GOS					Total	P-value
		Dead	Vegetative state	Severe disability	Moderate disability	Mild disability		
Agegroup/year	3-18	1	1	1	1	5	9	
	19-34	6	3	7	7	17	40	
	35-50	1	1	0	1	8	11	
	51-66	2	0	0	2	14	18	0.029
	67-82	0	1	0	0	0	9	
	83-98	3	0	0	1	8	2	
Total		12	5	9	11	51	89	

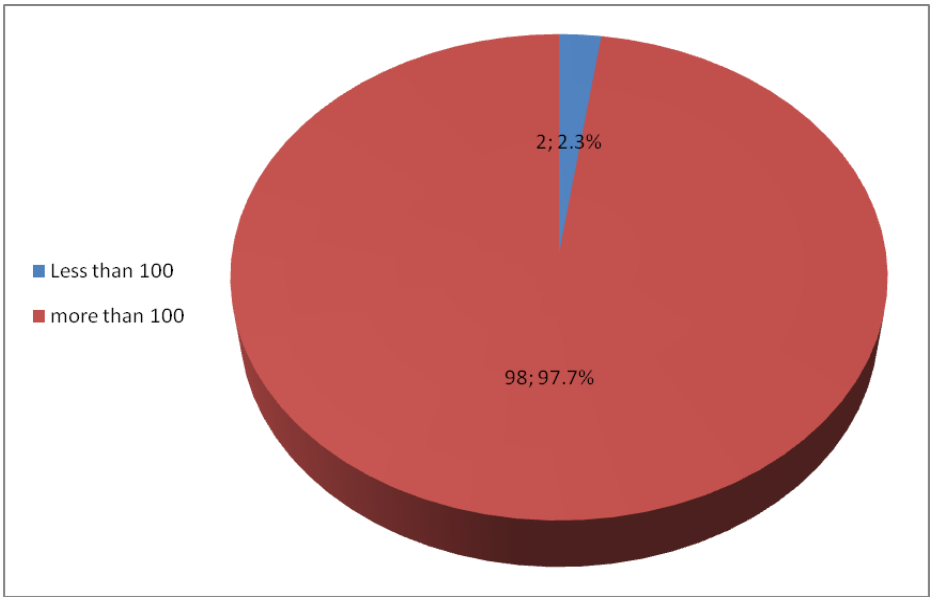

Figure 1: the frequency distribution of the level of S-100 B protein among patients.

The p-value of the goodness of fit using chi-square test P=.000.

Table 7: shows cross tabulation between computed tomography findings and S-100B protein.

Computed Tomography Findings	S-100 B protein					
	100-149 ng/L	150-199 ng/L	200-249 ng/L	250-300 ng/L	>300 ng/L	Total (%)
Brain edema	1	3	0	1	1	**6(6.9)**
Hemorrhagic Contusion (HC)	5	6	1		4	**16(18.4)**
axonal injury	1	3	2	0	0	**6(6.9)**
Extra dural heamatoma(EDH)	4	8	0	0	1	**13(15)**
Subdural heamatoma(SDH)	6	9	2	2	1	**20(23)**
Edema + HC	3	1	0	0	0	**4(4.6)**

Bullet injury	0	1	0	0	0	**1(1.1)**
SDH+HC	0	2	0	0	1	**3(3.5)**
Subarachnoid Hemorrhage (SAH)	2	2	1			**5(5.7)**
EDH+SDH+HC	1	1	0	0	1	**3(3.5)**
SAH +edema	1	0	0	0	0	**1(1.1)**
HC+SAH	1	1	0	0	0	**2(2.4)**
EDH+HC	0	1	0	0	1	**2(2.4)**
Normal	0	1	0	0	0	**1(1.1)**
H.C+SDH+Edema	0	1	0	0	0	**1(1.1)**
Epidural Hemorrhage	0	1	0	0	0	**1(1.1)**
axonal injury +Edema	0	1	0	0	0	**1(1.1)**
Edema+SAH+HC	1	0	0	0	0	**1(1.1)**
Total	**26(29.8%)**	**42 (48.3%)**	**6 (6.9 %)**	**3 (3.5%)**	**10(11.5%)**	**87(100%)**

Table 8: shows cross tabulation between GCS and S-100B protein.

GCS	**S-100 B protein**					
	100-149 ng/L	**150-199 ng/L**	**200-249 ng/L**	**250-300 ng/L**	**>300 ng/L**	**Total (%)**
Mild	18	27	3	2	5	55 (65.5%)
Moderate	4	11	2	1	5	23 (24.2%)
Severe	4	4	1	0	0	9 (10.3)
Total (%)	26(29.8%)	42 (48.3%)	6 (6.9 %)	3 (3.5%)	10(11.5%)	87(100%)

Table 9: shows cross tabulation between S-100B protein and the outcome of TBI among patient group.

GCS	**S-100 B protein**	**GOS**					**Total N (%)**
		Dead N	**Vegetative state N**	**Severe disability N**	**Moderate disability N**	**Mild disability N**	
Mild	100-149	2		1	2	13	18(20.7)
	150-199	1				26	27(31)
	200-249					3	3(3.4)
	250-300					2	2(2.3)
	>300					5	5(5.7)
Moderate	100-149	1		1		2	4(4.6)
	150-199	1		4	6		11(12.7)
	200-249	1			1		2(2.3)
	250-300			1			1(1.2)
	>300	1		2	2		5(5.7)
Severe	100-149	1	3				4(4.6)
	150-199	3	1				4(4.6)
	200-249	1					1(1.2)
	250-300						0(0)
	>300						0(0)
Total N (%)		12(13.8)	4(4.6)	9(10.3)	11(12.7)	51(58.6)	87(100)

DISCUSSION

TBI poses a real social and medical impact that is on increase worldwide.[20] TBI is the leading cause of death and disability, especially among the young population. Prognosis of patient's outcome after 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.[20]

Age is strongly associated with the outcome as shown in Husson, Murray, Mushkudiani, and Hebb studies; increasing age was associated with worse outcomes.[21,22,23,24] In our study, there is a significant association between age group with outcome after traumatic brain injury, 17/40 patients with age group (19-34 years) was associated with only mild disability (P=0.029). Studies were done by Gomez, Bricolo, Signorini, and Vollmer noted varied age thresholds (35, 40, 50, and 55 respectively) for poor outcome after TBI.[25,26,27,28] While Braakman, Heiskanen, noted 60 years as the critical threshold.[29,30,31] Moreover, a study done by Narayan showed that 57% and 78% of an unfavorable outcome in the age groups 41-60 and above 60 years, respectively.[6] Other study showed that worsening of outcome centered around 30-50 years and

there had a poorer outcome at 6 months among children less than 10 years of age.[6]

Proteomic biomarkers are a part of mainstream clinical care used to quantitatively assess and define injury in almost all organs system, except the brain.[14.32] In the absence of specific prognostic tools, there is increasing interest in identifying biomarkers that are both sensitive and specific to brain injury. S-100B proteins are one of the most recent biomarkers that aid in diagnosis and prognosis for individuals sustaining traumatic brain injury.[32]

S-100B belongs to a family of intracellular calcium-binding proteins predominantly present in mature perivascular astrocytes, but is also present to some extent in other cells in the central nervous system, including oligodendrocytes, neural progenitor cells and certain neuronal populations.[12,13] There are at least 25proteins that have been known as belonging to the S100 protein family.[10,33,34] S100B functions as a neurotrophic factor by promoting neurite outgrowth and astrocytic proliferation that results in increased neuronal function.[33] Elevated S100B levels after severe brain injury may reflect ongoing structural damage and cell death. Numerous studies have demonstrated increased S100B levels, significantly above control levels, after severe TBI in both CSF and serum.[34, 35, 36]

In an experimental study done by Ciccarelli and others demonstrated that S100B is a secretion from astroglia within a few minutes after receptor activation, and release may last up to 10 h.[37,38] Although injury-induced S100B release continues to increase up to 48 h in cell cultures.[39,40] S100B serum levels in patients are highest directly after the injury and become normalized within 24 h in a high percentage of cases (even in those patients with a bad outcome).[41]

This is the first study to quantitatively examine the serum S-100B level in TBI patients in Sudan. The samples were collected in a period of post-trauma ranging between 24 hours to 6 days. The results in this study showed that S-100 B protein significantly increased in patients with TBI. P= 0.00.

Studies were done by Kleindienst, Goyal, Bohmer, and Schmidt showed that CSF and serum S100B concentrations were significantly increased for 6 days above control levels in patients after severe TBI.[42, 43, 44, 45] Kleindienst demonstrated S100 B protein levels in CSF and serum daily for 10 days in TBI patients and controls and the results showed that S100B levels were significantly increased in the serum on day 1 to 8 and in the CSF on day 1 to 10.[42]

In resting cells, S100B proteins are localized in specific cellular compartments from which some of them relocate upon cellular stimulation and even are secreted extracellular as cytokine-like activity, this translocation might be a temporal or spatial determinant of their interactions with different partner proteins, this is supported by a study done by Romner B that showed all individuals in the control group showed a non detectable serum level of S-100 protein. Among the head-injured patients, the protein was detectable in 108 (39%) and nondetectable in 170 (61%) when the sample was drawn within 24 hours following injury and the detectable serum S100 protein level of at least 0.2 µg/L.[46]

Moreover, S-100B is a more reliable marker of the severity of the primary injury that causes disruption of the blood-brain barrier through which brain-septic markers are released into the bloodstream. The early concentration peak of these markers reflect the mechanical disruption of brain tissue.18. In our study, a level of S-100 B 150-199 ng/L was expressed by 27 patients with GCS (mild), and 11 patients of moderate and there is only 4 patient with severe GCS. In addition to that, taking the level of this marker and radiological Computed tomography finding we showed that 9 (45%) of the patients with Subdural hematoma were associated with S-100-B level of 150-199 ng/L. ((At this level of S-100 B, 8 (62 %), 6 (37.5 %), 3 (50%), 3 (50%) of the patients were associated with Extradural hematoma, Hemorrhagic contusion, Brain edema, and Axonal injury respectively. While at the S-100 B level of > 300 ng/L was detected in 10 patients with hemorrhagic contusion, brain edema, extradural hematoma, and subdural hematoma, the higher percentage 40% (4 out of 10), was found in patients with Hemorrhagic contusion)) percentages do not reflect the correlation, it might be more appropriate to look at the level of S100 protein in each category separately and then discuss the results if there is a significant result. The previous studies discuss the relationship between GCS, CT findings, and S-100 B levels. Herrmann et al and Raabe et al demonstrated a positive correlation between the S-100B levels in serum and GCS, CT scan findings, and volume of brain contusions.[47,48] In Sweden, Calcagnile and others Added S-100B to existing guidelines for mild traumatic brain injury after study the actual cost with S-100 B measurement and without measurement of these protein and concluded that S-100 B seems to reduce CT usage and costs in which among patients with normal S100B levels, none of these showed any intracranial complications.[49] Another study done by Abbasi found that serum S-100 B increased in mild to moderate traumatic brain injuries[50] but the time of sample collection within the first six hours of their head injury and is differ from our study .while a study done by Mehta found that raised serum level of S-100B proteins associated with low GCS.[51]

In this study S100 B levels of 150-199 ng/L was significant in mild GCS, 26/27 (96%)and associated with mild disability while in moderate GCS, 6/11 (55%) of these patients were associated with moderate disability, 4/11(36%), severe disability and death 1/11(9 %). Moreover, only one patient with S100 B level of 150-199

ng/L and severe GCS was associated with vegetative state although about 3 patients with the same GCS and GOS were associated with S100 B level of 100-149 ng/L. Mehta reported that patients with good outcome and moderate disability demonstrated a similar pattern of S-100B levels, with high initial values and no secondary increase. Initial values were clearly associated with the outcome; higher initial values were associated with moderate disability, and lower values were associated with good outcome. Because S-100B has a half-life of 30 minutes, initially increased values of S-100B released by the primary damage should return to baseline levels rapidly unless there is ongoing damage.[51] Studies done in Iran highlighted the role of blood-brain barrier integrity and disruption in S100B release into the serum, the possible roles of S100B that may improve outcomes in patients with acute brain injuries. These studies also reported a relationship between S100B values and likely outcomes in patients with TBI.[50] A large cohort study showed some association between high serum level and poor outcome in patients with brain injury, but not significant to support use as an outcome prediction tool.[41] Metting et al. studied 94 patients with mild TBI and reported that S-100 B is not related to outcome or imaging results.[52] Some newer studies have proposed that serum S-100 B level might be used for predicting the probability of brain death in patients with TBI.[50]

CONCLUSIONS

The findings of this study showed an association between age and outcome after TBI and a association between S-100 B protein level>150 ng/L and outcome after TBI but not significant to use this marker as prognostic tool, to support these association we proposed further big study and linking S100b levels to other degenerative biomarkers like amyloid precursor protein and presenilin to consider the utility of this marker in tracking long-term chronic conditions associated with TBI and ongoing brain distress.

ACKNOWLEDGMENT

This study was conducted at the research laboratory in the National Center for Neurological Sciences (NCNS) Sudan. Authors would like to thank the Center and their patients for participation. This study is a part of the research for the fulfillment of Ph.D. degree for the student Salma Hussein Abdalla Elhassan from Al-Neelain University, Sudan in collaboration with the National Center for Neurological Sciences, Sudan.

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