ORIGINAL ARTICLE

The diagnostic accuracy of serum and urinary S100B protein in children and adolescents with mild traumatic brain injury

Javad Mozafari, Mohammad Ali Fahimi, Kourosh Mohammadi, Hassan Barzegari, Mohammad Ghasem Hanafi and Amal Saki-Malehi

ABSTRACT

Objective: To determine the role of S100B as a serum and urinary biomarker in children with mild traumatic brain injury.

Methods: A cross-sectional study in children and adolescents (6 months to 18 years) with mild traumatic brain injury who were referred to the Emergency Department of Golestan Hospital, Ahvaz City, Iran. The patients were divided into two groups after a brain CT-scan: Group A with positive pathological findings and Group B with a normal brain CT scan. Serum and urinary levels of S100B biomarker were compared between the two groups.

Results: A total of 40 children and adolescents were evaluated in patient Groups A and B (20 in each group). The area under the ROC curve was 0.998 (P <0.0001) which indicated a high precision level of serum S100b in the differentiation between the two groups, the best cut-off point was 172.15 ng/l with 95% sensitivity and 100% specificity. In addition, the area under ROC curve of 0.985 (P <0.0001) indicated a high accuracy for S100B urinary concentrations to differentiate between the two groups, the best-obtained cut-off point was 67.75 with a sensitivity and specificity of 90% and 95 % respectively.

Conclusion: The results of this study demonstrated that use of serum and urinary levels of the S100B biomarker can reduce unnecessary brain CT scans in children and adolescents with mild traumatic brain injury. Considering the non-invasiveness of the urinary sample collection, this method can be used in an emergency instead of serum.

Key words: brain injury, biomarkers, S100B protein, diagnosis, emergency service.

N Z J Med Lab Sci 2019; 73: 96-99

INTRODUCTION

Approximately 15% of patients with mild traumatic brain injury with Glasgow coma scale scores of 14 and 15 (classified as mild) from their brain computerised tomography scan (CT scan) have pathologies, such as subarachnoid haemorrhage, skull fracture, intracranial subdural and epidural haemorrhages, of which less than 1% need neurosurgery intervention. According to the available guidelines to determine the presence and extent of brain damage, (depending on clinical symptoms of the patient), a standard brain CT scan is administered in this group of patients. However, this imaging modality is expensive and time consuming and importantly, may increase the risk of cancer due to exposure to ionising radiation (3,4).

The S100 calcium-binding protein B, belongs to the family of Ca^{2+} binding proteins (5) and helps to regulate the amount of intracellular calcium (6). This protein has been identified in high concentrations in Schwann and Astrocytes cells (7) and has also been identified in other tissues, such as bone marrow cells, lymphocytes, adipocytes and melanocytes (8-10). S100B has the highest concentrations in the cerebrospinal fluid and serum, however, this protein can also be detected in other body fluids, including amniotic fluid, urine and cord blood (11). Based on previous research the initial level of S100B at 3 and 6 hours after a mild traumatic brain injury can be considered as a biomarker in predicting some outcome-related events as there is a correlation between CT scan pathological findings and a high level of S100B (12).

The aim of this study was to determine the concentration of serum and urine levels of the S100B as a biomarker and compare them with mild brain trauma in children and adolescents, to provide an acceptable estimate of the presence or absence of positive and negative findings of brain CT scans.

METHODS

Study design

This cross-sectional study was approved by the Jundishapur University of Medical Sciences Ethic Committee and parents/ guardians were approached for informed consent for children and adolescent with head injuries referred to the Emergency Department of the Ahvaz Golestan Hospital, Iran during April to September 2017. This study has been conducted according to the Standards for Reporting Diagnostic Accuracy (**STARD**) (14)**.**

Participants

All children and adolescent with diagnosis of mild traumatic brain injury who met the inclusion criteria were entered into this study. The inclusion criteria were the presence for an indication of a brain CT scan, aged 6 months to 18 years and a Glasgow coma score of 14 or 15. Injuries included those from traffic and home or sport events, and referrals less than 6 hours of the incident. Inclusion criteria were no previous history of alcohol or drug abuse, the absence of a history of previous neurological disease such as seizure or epilepsy, the absence of severe traffic injury and multiple trauma from motor vehicles, and absence of melanoma. Patients were excluded from the study if they had any of the following conditions: injuries except the brain mild trauma damage such as organ damage, previous illnesses such as diabetes, heart disease, asthma, pregnancy or recent febrile illness.

Test methods

Following initial examination and stabilisation, venous blood and urine samples were obtained by the attending nurse and the patients were then referred to a fixed imaging unit to obtain a skull CT scan. Primary information was recorded about the nature of injury and the presence of ligation, scratching,

ontogeny, and size and location of lesion in the scalp and face, Glasgow coma score, headache, nausea, vomiting, dizziness, neurological deficits, amnesia and decreased consciousness and its duration. The blood and urine samples were immediately transferred to the central laboratory of Golestan Hospital. Blood samples were centrifuged at room temperature for 10 minutes at 2200 g and the separated serum was stored at -70 ° C until analysis. Urine samples were centrifuged at room temperature for 10 minutes at 900 g and the supernatant was stored at -70° C until analysis. The S100B in both serum and urine samples were determined using S100B ELISA kits (Shanghai Crystal Day Biotech Co., Ltd). The concentration of S100B in serum and urine of each sample was recorded independently without knowledge of the brain CT scan results. The primary brain CT scans of all patients was interpreted using a 64 slice CTscan device and independently interpreted by a consultant neurologist who was not aware of the results of the corresponding S100B results. Patients were assigned to either Group A or Group B according to their CT scan results. **Data analysis**

Results from both the urine and serum concentrations of S100B and the results of the interpretation of brain CT scans were analysed by an independent t-test and the Mann-Whitney U test (SPSS 22). The area under the curve (AUC), sensitivities and specificities of the data were determined. The level of statistical significance was set at $p<0.05$.

RESULTS

Participants

In total 40 participants were evaluated, 20 who had positive pathologic findings associated with isolated head trauma (Group A) and those who lacked these findings in brain CT scans (Group B). The frequency of patients and their age distribution and the characteristics of both groups in brain CT scans are presented in Table 1.

Table 1. Patient's characteristics.

Variables		Group A (N= 20)	Group B (N= 20)
Median age in years (range)		9 (2-18)	6.6 (0.5 - 18)
Female N (%)		4 (20)	8 (40)
GCS (%)	14 score GCS (%)	4 (20)	13 (65)
	15 score GCS (%)	16 (80)	7 (35)
Average admission time after trauma in hours		2.79	2.66

Serum and urine S100B

In Group A the mean (±1SD) serum level of S100B was 561±283 ng/L, whereas in Group B it was 79.8±22.8 ng/L ($p<0.001$) (Table 2). A serum level of 172.15 ng/L had a sensitivity, specificity, PPA, and NPA of 95%, 100%, 100%, and 91% respectively for the diagnosis of intra cerebral lesions according to positive findings of the brain CT scan ($p<0.0001$). In group A, the mean urinary level of S100B was 134±63.5 ng/L, whereas in group B it was 25±19 ng/L ($p<0.001$) (Table 2). Urinary S100B levels of 67.75 and 56.4 ng/L with a sensitivity and specificity of 90% and 95% and 95% and 90% respectively were used to estimate CT scan results. There was a significant difference between the serum and urinary levels of S100B between the two groups and therefore had the potential to differentiate between these two groups of patients.

The area under the ROC curve of 0.998 ($P<0.0001$) indicated a high predictive value of serum S100B in the differentiation between positive and negative patients. The cut-off point of 172.15 ng/L with a sensitivity of 95% and a specificity of 100% was the best cutting point in the area under the ROC curve.

The area under the ROC curve with a value of 0. 985 ($P<0.0001$) indicated a high accuracy of the urine S100B level in differentiating between positive and negative patients. Considering the area under curve, the cutting point was 67.75 with a sensitivity of 90% and specificity of 95%, and at the cutting point of 56.4 a sensitivity of 95% and specificity of 90%.

Table 2. S100B levels and clinical signs.

Variables	Group A (n= 20)	Group B (n= 20)	P
Urinary S100B ng/L Mean ± SD	134.59 ± 63.53	25.08 ± 19.32	<0.001
Serum S100B ng/L Mean ± SD	561.53 ± 283.37	79.83 ± 22.85	<0.001
Headache n (%)	9 (45)	12 (60)	0.9
Nausea & vomiting n (%)	13 (65)	10 (50)	
Confusion n (%)	1 (5)	2 (10)	
Functional neuro-logical disorder n (%)	1 (5)	0	
Vertigo n (%)	3 (15)	0	

DISCUSSION

Traumatic brain injury is one of the most common clinical complaints that lead to referral to the emergency department. Since most of the complications of head injury occur over passage of time, early detection of those who are likely to show these lesions plays a very important role in determining the clinical outcome for these patients. According to existing methods, brain CT scans detect brain damage in people with symptoms such as vomiting, however, this imaging method is expensive and not readily available in some centres and most importantly, most people who undergo a brain CT scan do not show any initial positive findings.

Considering our findings, when a child with mild traumatic brain injury is referred during the first 6 hours after the incident, serum or urinary levels of the S100B biomarker have an acceptable sensitivity and specificity for selecting patients requiring a definite brain CT scan. This approach could prevent unnecessary CT scans with negative findings, clinical risk of exposure to ionising radiation, reduce costs and stay in the Emergency Department. On the other hand, due to the non-invasive nature of the urine sampling and no significant difference between serum and urinary S100B levels in determining the presence and extent of brain CT scan, urinary samples may be considered as a non-invasive and more convenient method than serum samples.

Findings of our study showed that serum and urinary levels of S100B could significantly contribute to the positive pathological findings in brain CT scans, whereas no significant difference was observed between serum and urinary S100B levels. Previous studies have demonstrated the relationship between high serum levels of S100B and the presence of positive pathological findings in brain CT scans. Although these studies have been conducted in adults (14-17), studies on children and adolescent are few and studies that show the association between the urinary levels of this biomarker and positive findings of brain CT scans are scarce (14,18,19). Hallen *et al.*

did not show that urinary values of this biomarker increases during the first six hours of mild traumatic brain injury (20). Varying serum levels S100B, ranging from 100-240 ng/L, have been reported in studies with different sample sizes that should be interpreted according to the positive or negative predictive values and the sensitivity and specificity of the obtained cutoff values (21). In our study, the subjects who had positive findings in brain CT scans were all healthy before the recent incident and they did not have any effect on the levels of the biomarker, including multiple trauma, or symptoms such as rapid breathing or fever, and several cases such as head previous trauma and congenital toxoplasmosis.

In our study, a S100B serum level of 172.15 ng/L had a sensitivity, specificity, PPA, and NPA of 95%, 100% , 100%, and 91% respectively in the diagnosis of intra cerebral lesions and with the positive findings of brain CT scans ($p < 0.0001$). We have shown that a S100B urine level of 67.75 ng/L had a sensitivity and specificity of 90% and 95% respectively and a level of 56.4 ng/L had a sensitivity and specificity of 95% 90% respectively, in agreement with the positive findings of the CT scan.

Hallen *et al.* studied six children and adolescents with reported CT scan positive findings. The mean serum S100B level in the group that immediately underwent a brain CT scan was 111 ng/L after admission, range: 86-153ng/L (20). They showed that a serum S100B cut-off value of 195 ng/L with a sensitivity and specificity of 100% and 88% respectively, was consistent with CT scan findings. They also showed that urinary levels of S100B did not correlate with brain CT scan findings, which are acquired during the first 6 hours after trauma incident. Their study, however, showed a correlation between serum levels S100B levels and brain CT scan findings and these values were significantly helpful with prediction of CT scan findings (20).

Our choice of a 172.15 ng/L cut-off value for serum S100B is higher than the cut-off value of the Castellani *et al.* study (22). The differences in the values can be attributed to the severity and diversity of pathologic findings (22) as well as the differences in the manufacturing companies of the analytical kits.

Contrary to our study, few studies on the possible correlation between urinary S100B levels and positive brain CT scans have been previously conducted in children and adolescents. However, since findings of these studies showed no correlation between them, it is essential to conduct studies on urine specimens with a larger number of patients.

The average time for taking samples (urine and serum) was 2.79 hours in our study, while in the Hallen *et al.* study it was 3.5 hours. Due to the short half-life of this biomarker, this may explain the correlation with the positive brain CT scan in our study compared to the Hallen *et al.* study.

However, this biomarker may also be increased in other cases, such as brain infections (23). In our study, false positives and false negative were considered and were not included in the study from the very beginning.

In summary, previous studies have established a correlation between urinary S100B levels and positive findings of brain CT scans, however, few studies have been conducted in children and adolescents (21,24). Most of these studies reported no significant correlations. Our findings have demonstrated a significant relationship between serum and urinary S100B levels with positive findings of brain CT scans and further studies are required with a larger sample size of urinary samples. Our study had some limitations, namely patient follow-up was not possible and the sample size was small.

In conclusion, results of our study showed that the use of serum and urinary levels of the S100B biomarker could reduce unnecessary brain CT-scans in children and adolescents with mild traumatic brain injury. Considering the non-invasiveness of the urinary sample collection, this method can be used in an emergency instead of serum samples.

ACKNOWLEDGMENT

The authors wish to acknowledge the support of the deputy of research affairs of the Ahvaz Jundishapur University of Medical Sciences as part of Kourosh Mohammadi's thesis under the research code: U-94138.

AUTHOR INFORMATION

Javad Mozafari, MD, Assistant Professor of Emergency Medicine[1]
Mohammad Ali Fahimi, MD, Assistant Professor of Emergency Medicine[1]
Kourosh Mohammadi, MD, Assistant Professor of Emergency Medicine[1]
Hassan Barzegari, MD, Assistant Professor of Radiology[2]
Mohammad Ghasem Hanafi, MD, Professor of Radiology[2]
Amal Saki-Malehi, PhD, Assistant Professor of Biostatistics and Epidemiology[3]

[1]Department of Emergency Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2]Department of Radiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[3]Department of Biostatistics and Epidemiology, Faculty of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Corresponding author: Mohammad Ali Fahimi.
Email: fahimi-m@ajums.ac.ir

REFERENCES

1. Morton MJ, Korley FK. Head computed tomography use in the emergency department for mild traumatic brain injury: integrating evidence into practice for the resident physician. *Ann Emerg Med* 2012; 60: 361-367.
2. Walls R, Hockberger R, Gausche-Hill M. Rosen's Emergency Medicine-Concepts and Clinical Practice E-Book (7th Edition), 2017. Elsevier Health Sciences.
3. Andrade FP, Montoro R Neto, Oliveira R, Loures G, Flessak L, et al. Pediatric minor head trauma: do cranial CT scans change the therapeutic approach? *Clinics* 2016; 71: 606-610.
4. Asl JF, Shirbandi K, Naghashpour M, Mehr FJ. Medical interns' and residents' awareness of radiation protection in pediatric diagnostic imaging. *Jentashapir J Health Res* 2018; 9(3): e66932.
5. Zimmer DB, Cornwall EH, Landar A, Song W. The S100 protein family: history, function, and expression. *Brain Res Bull* 1995; 37: 417-429.
6. Astrand R, Undén J, Romner B. Clinical use of the calcium-binding S100B protein. *Methods Mol Biol* 2013; 963: 373-384.
7. Zuo Y, Lubischer JL, Kang H, Tian L, Mikesh M, Marks A, et al. Fluorescent proteins expressed in mouse transgenic lines mark subsets of glia, neurons, macrophages, and dendritic cells for vital examination. *J Neurosci* 2004; 24: 10999-11009.
8. Faye RS, Paus E, Maelandsmo GM, Berner A, Hoifodt HK, Fodstad O, et al. S100B in bone marrow aspirates in healthy individuals and malignant melanoma patients. *Melanoma Res* 2008; 18: 134-140.
9. Diaz-Romero J, Kürsener S, Kohl S, Nesic D. S100B + A1 CELISA: a novel potency assay and screening tool for redifferentiation stimuli of human articular chondrocytes. *J Cell Physiol* 2017; 232: 1559-1570.
10. Cheong KA, Noh M, Kim CH, Lee AY. S100B as a potential biomarker for the detection of cytotoxicity of melanocytes. *Exp Dermatol* 2014; 23: 165-171.

11. Wainwright MS, Craft JM, Griffin WS, Marks A, Pineda J, Padgett KR, et al. Increased susceptibility of S100B transgenic mice to perinatal hypoxia-ischemia. *Ann Neurol* 2004; 56: 61-67.
12. Rezaei O, Pakdaman H, Gharehgozli K, Simani L, Vahedian-Azimi A, Asaadi S, et al. S100 B: A new concept in neurocritical care. *Iran J Neurol* 2017; 16: 83-89.
13. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. *BMJ* 2015; 351: h5527.
14. Berger RP, Kochanek PM: Urinary S100B concentrations are increased after brain injury in children: A preliminary study. *Pediatr Crit Care Med* 2006; 7: 557-561.
15. Zongo D, Ribéreau-Gayon R, Masson F, Laborey M, Contrand B, Salmi LR, et al. S100-B protein as a screening tool for the early assessment of minor head injury. *Ann Emerg Med* 2012; 59: 209-218.
16. Kotlyar S, Larkin GL, Moore CL, D'Onofrio G. S100b immunoassay: an assessment of diagnostic utility in minor head trauma. *J Emerg Med* 2011; 41: 285-293.
17. Calcagnile O, Undén L, Undén J. Clinical validation of S100B use in management of mild head injury. *BMC Emerg Med* 2012, 12: 13.
18. Manzano S, Holzinger IB, Kellenberger CJ, Lacroix L, Klima-Lange D, Hersberger M, et al. Diagnostic performance of S100B protein serum measurement in detecting intracranial injury in children with mild head trauma. *Emerg Med J* 2016; 33: 42-46.
19. Oris C, Pereira B, Durif J, Simon-Pimmel J, Castellani C, Manzano S, et al. The biomarker S100B and mild traumatic brain injury: A meta-analysis. *Pediatrics* 2018; 141: e20180037.
20. Hallén M, Karlsson M, Carlhed R, Hallgren T, Bergenheim M. S-100B in serum and urine after traumatic head injury in children. *J Trauma* 2010; 69: 284-289.
21. Thelin EP, Nelson DW, Bellander BM. A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. *Acta Neurochir (Wien)* 2017; 159: 209-225.
22. Castellani C, Bimbashi P, Ruttenstock E, Sacherer P, Stojakovic T, Weinberg AM. Neuroprotein S-100B - a useful parameter in paediatric patients with mild traumatic brain injury? *Acta Paediatr* 2009; 98: 1607-1612.
23. Undén J, Christensson B, Bellner J, Alling C, Romner B. Serum S100B levels in patients with cerebral and extracerebral infectious disease. *Scand J Infect Dis* 2004; 36: 10-13.
24. Schulte S, Podlog LW, Hamson-Utley JJ, Strathmann FG, Strüder HK. A systematic review of the biomarker S100B: implications for sport-related concussion management. *J Athl Train* 2014; 49: 830-850.