FULL PAPER

Correlation of Neuron Specific Enolase protein levels with the severity of traumatic brain injury as measured by the Glasgow Coma scale and marshall classification

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Traumatic Brain Injury (TBI) is a trauma with high morbidity and mortality. The degree of TBI severity so far uses the Glasgow Coma Scale (GCS). Marshall classification of traumatic brain injury from head CT scans has been shown to predict outcome in patients with traumatic brain injury. Neuron Specific Enolase (NSE), a potential biomarker of brain damage for prognostic evaluation, is a structural protein of the central nervous system, which will be regulated to maintain homeostasis in the event of axonal damage. This study aims to find a relationship between Neuron Specific Enolase protein levels and the severity of traumatic brain injury as measured by the Glasgow Coma Scale and Marshall classification. Observational, cross-sectional study with a sample of 51 subjects who were diagnosed with traumatic brain injury and admitted to the emergency room. The subject was resuscitated, a blood sample was taken to check NSE levels and a head CT scan was examined. The research was conducted at Dr Soetomo Hospital, Surabaya from August to October 2023. Fifty-one subjects in this study, consisting of 36 men and 15 women, with an average age of 43 years. The results show that the lower GCS is strongly correlated with the higher NSE value with the correlation test results r = -0.467, p < 0.001. The results show that the higher the Marshall classification is strongly correlated with the higher the NSE value with the correlation test results r = 0.475, p < 0.001. There is a significant relationship between NSE levels and GCS levels and also with higher the NSE level, the GCS is decreased. Likewise, there is a significant relationship between the NSE level and the Marshall classification, the higher the NSE level, the higher the Marshall classification.

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Introduction

Traumatic brain injury, or intracranial injury, is an injury to the brain caused by external forces [1]. TBI is classified based on severity (mild, moderate, and severe), mechanism (closed or penetrating), and other features (such as the area of the brain affected or the

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name of the brain part) [2]. Mechanical damage that occurs during impact is called primary injury, and then secondary injury occurs where sequential pathological processes occur with delayed clinical symptoms, such as cerebral ischemia and intracranial hypertension, which are sensitive to therapeutic intervention [3,4].

Increasingly advanced technology, both in the world of medicine and biochemistry, has encouraged many discoveries to support clinical trials. Biomarkers of brain damage have been known for a long time and their use is starting to become widespread, one of which is Neuron Specific Enolase (NSE). NSE is a structural protein of the central nervous system, located mainly in the cytoplasm of neurons, which participates in slow axoplasmic transport [5]. Under normal circumstances, NSE is not secreted, but if axons are damaged, NSE is regulated to maintain homeostasis, so it can be used directly to assess functional damage to neurons [6].

The severity of traumatic brain injury is measured based on structural features, loss of consciousness, changes in mental status, post-traumatic amnesia, and the Glasgow Coma Scale, into three categories, namely mild, moderate and severe traumatic brain injury [7,8]. The Glasgow Coma Scale itself is a practical method for assessing decreased consciousness in response to specified stimuli, and is used in clinical practice to this day [9]. Assessment is based on eye opening which is given a score of 1-4 (ranging from not opening the eyes at all, opening after a pressure stimulus, opening after a sound stimulus, and opening spontaneously), verbal reaction with a score of 1-5 (ranging from no verbal reaction at all, only groans, only utters a few words, confused, and speaks fluently), and movement reactions with a score of 1-6 (no movement at all, abnormal extension, abnormal flexion, normal flexion with stimulation, localizes pain, and obeys order) [10].

The Marshall system is a patient selection management based on the severity of non-contrast head CT scan findings. The principle is the degree of swelling and the presence of bruising or bleeding. The classification is: 1) diffuse injury I = no visible intracranial pathology, 2) diffuse injury II = midline shift of the brain up to 5 mm, basal cisterns remain visible, no lesion > 25 cm^3, 3) diffuse injury III = line shift middle of the brain up to 5 mm, basal cisterns compressed or missing, no lesions > 25 cm^3, 4) diffuse injury IV = midline shift of the brain > 5 mm, no lesions > 25 cm^3, 5) mass lesion evacuated V = action has been taken surgery to evacuate the lesion, 6) non-evacuated mass lesion VI = lesion > 25 cm^3, not evacuated surgically [11,12].

Experimental

This study was observational with a cross-sectional design, aiming to determine the comparison between NSE protein levels and the severity of TBI as measured by GCS and Marshall classification. This research was conducted in the emergency room of Dr Soetomo Hospital Surabaya from August 2023 to October 2023. The inclusion criteria were: 1) all TBI patients with any GCS who came to the emergency room, 2) patients aged 17-65 years. Meanwhile, the exclusion criteria are: 1) the patient's family refuses to be included in the study, 2) patients with a history of Alzheimer's disease, DM, melanoma, multiple sclerosis, Down syndrome, and epilepsy.

Patients who met inclusion underwent a GCS examination after initial resuscitation, blood serum samples were taken for NSE examination, and a head CT scan was performed. The blood serum taken was processed in the clinical laboratory at Dr Soetomo Hospital Surabaya, and the NSE levels were checked using the ELISA method. The subject's head CT scan was assessed by a radiologist and his Marshall classification recorded.

Results

Fifty-one subjects were obtained in this study. Subjects were patients diagnosed with traumatic brain injury, either due to traffic accidents, household accidents, or work accidents. Subject characteristics can be seen in Table 1.

TABLE 1 Research characteristics

Characteristics	N	%
Sex		
Male	36	70.6%
Female	15	29.4%
Age (mean±SD) = 43.4±16.0 years		
< 20 years	5	9.9 %
20-29 years	7	13.8 %
30-39 years	10	19.6 %
40-49 years	10	19.6 %
50-59 years	6	11.8 %
60-69 years	13	25.5%
Action		
Craniotomy surgery	32	62.5 %
Non craniotomy surgery	19	37.5 %
End care's outcome		
Survival	36	70.6 %
Non survival	15	29.4 %
Classification of TBI based on GCS		
Mild – moderate (GCS > 8)	27	52.94 %
Severe (GCS ≤ 8)	24	47,06 %
Marshall Classification		
Low – intermediate (Diffuse Injury I – IV)	14	27.5 %
High (Evacuated Mass V – non evacuated mass VI)	37	72.5 %

Blood serum taken from 51 patients was examined for NSE using the ELISA method, and a mean value of 2.62 ng/mL with an SD of 0.89 was obtained, as presented in Table 2.

TABLE 2 NSE research result

Parameter	Mean	Median	Mode	SD	Value Range
NSE (ng/mL)	2.62	2.62	1.68	0.89	1.10- 4.66

The classification of TBI in this study was divided into two categories based on GCS, namely mild-moderate TBI (GCS > 8) and severe TBI (GCS ≤ 8). From these two categories, the Shapiro-Wilk method statistical normality test was carried out and the p-values were 0.685 and 0.828, respectively, indicating that the distribution was normal. The t test for each category shows significant differences (Figure 1), with the mild-moderate TBI category or GCS > 8 (N = 27) having a mean ± SD = 2.25 ± 0.72, while the severe TBI category or GCS ≤ 8 has a mean value ± SD = 3.04 ± 0.89, with a difference test having a p-value of 0.044. The Pearson correlation test for the relationship between NSE levels and TBI severity based on GCS had r = - 0.467 with $p < 0.05$ (Table 3).
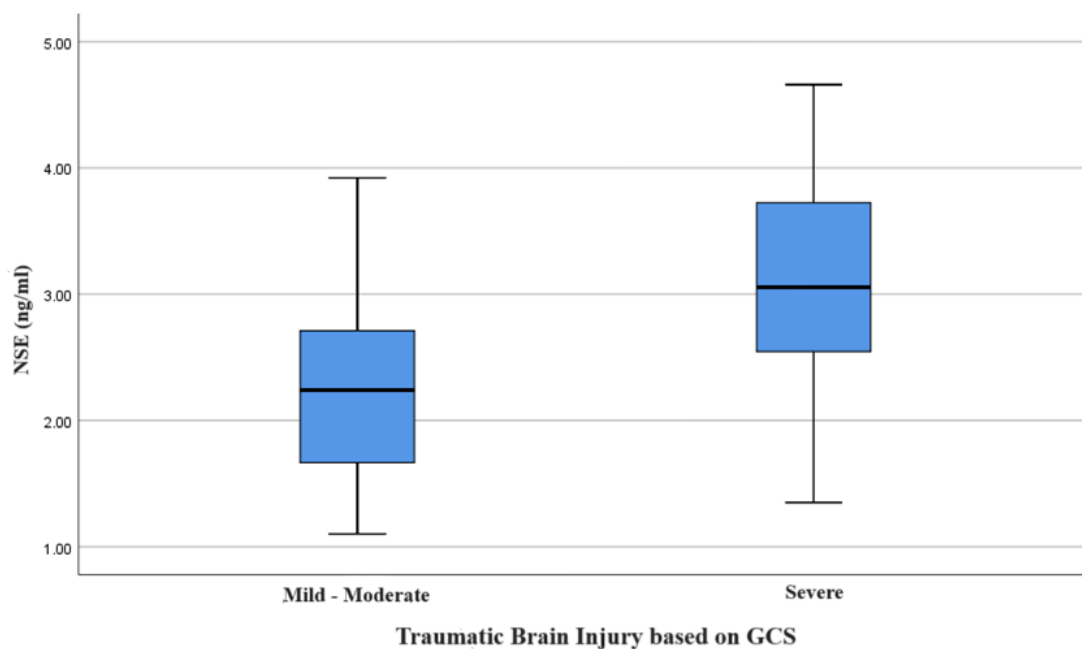
FIGURE 1 Comparison of NSE levels based on the severity of traumatic brain injury from GCS values

TABLE 3 Statistical test for the relationship between NSE levels and GCS

GCS	Mild – moderate TBI	Severe TBI
Normality test		
P-value (Saphiro-Wilk)	0.685	0.828
Difference test (p-value 0.044		
Mean ± SD (N)	2.25 ± 0.72 (27)	3.04 ± 0.89 (24)
Pearson correlation test, R = - 0.467 , p value 0.001		

The TBI classification based on the Marshall classification in this study is divided into two, namely the low- intermediate Marshall classification (diffuse injury I-IV) and the high Marshall classification (evacuated mass V – non evacuated mass VI). From these two categories, the Shapiro-Wilk method statistical normality test was carried out and the p-values were 0.077 and 0.909, respectively, indicating that the distribution was normal. The t test for each category shows significant differences (Figure 2), with the Marshall classification category low - intermediate or diffuse injury I - IV (N = 14) having a mean ± SD = 2.11 ± 0.91, while the High Marshall classification or evacuated mass V – non evacuated mass VI has a mean value ± SD = 2.82 ± 0.82, with a difference test having a p-value of 0.010. The Pearson correlation test for the relationship between NSE levels and Marshall classification has r = 0.475 with p < 0.05 (Table 4).
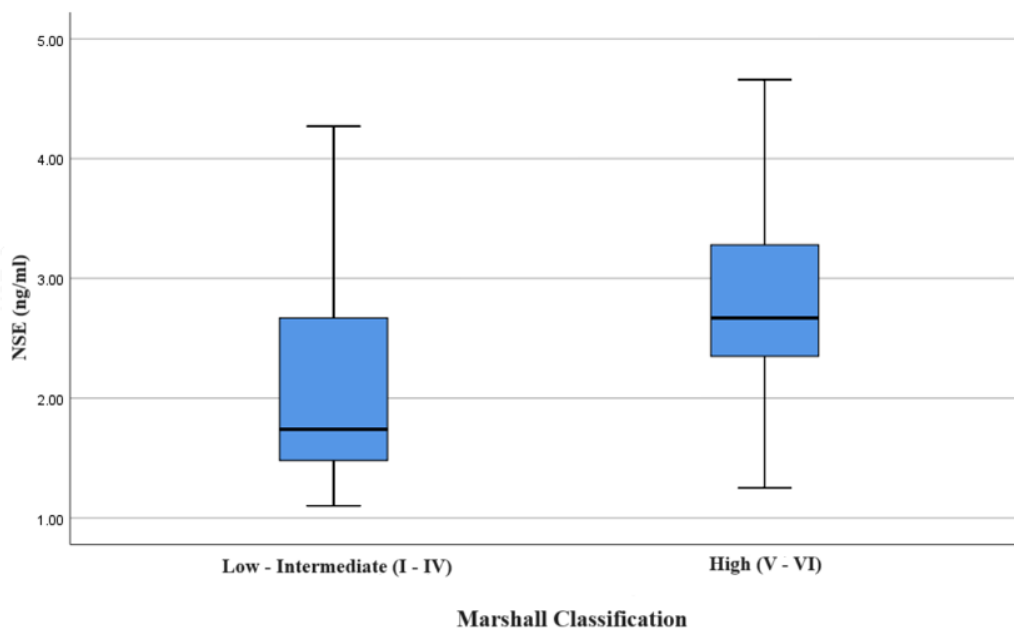
FIGURE 2 Comparison of NSE levels based on Marshall classification

TABLE 4 Statistical test for the relationship between NSE levels and Marshall classification

Marshall Classification	Low - intermediate	High
	Normality test	
p-value (Saphiro-Wilk)	0.077	0.909
	Difference test (p-value 0.010)	
Mean ± SD (N)	2.11 ± 0.91 (14)	2.82 ± 0.82 (37)
	Pearson correlation test, R = 0.475 , p value 0.000	

Discussion

Fifty-one subjects in this study showed that there was an increase in NSE levels in traumatic brain injury with levels of 2.62 ng/ml. Cheng's research in 2014 showed an increase in NSE in traumatic brain injury patients with levels of 0.96- 2.32 ng/ml [13].

This study shows that there is a correlation between the NSE level and the patient's GCS at admission (after initial resuscitation), namely the higher the NSE level, the lower the GCS. Zaheer *et al.* found something similar, but in acute ischemic stroke patients, where they concluded that serum NSE levels were useful as a marker for predicting stroke severity and early functional outcomes [14].

Richter et al in 2022 conducted research on patients with injuries resulting from trauma and found that a high Marshall classification was associated with increased NSE levels [15]. This research concludes the same thing, where the higher the NSE level, the higher the Marshall classification. NSE as a protein in the cytoplasm of neuronal cells, and S100B, correlate with radiological measurements of infarct volume in the first week of stroke.

Biomarkers of brain damage have been widely discovered, starting from Neuron Specific Enolase, S100B, Glial Fibrillary Acidic Protein, Myelin Basic Protein, and tau protein [16]. In the early phase of acute stroke, biomarker determination is cheaper and easier to measure tissue damage and evaluate treatment effects than other modalities, such as radiology [17]. The hope for TBI is that biomarkers of brain damage can also show the same thing, especially in improving diagnosis and evaluation.

The limitation of this study is that it did not examine post-procedure NSE and carry out periodic NSE evaluations so that we could see trends in NSE during treatment. Multivariate

analysis could not be carried out because the determining factor for increased NSE was only due to trauma, there were no other variables such as comorbidities, history of previous trauma, type of trauma whether isolated brain injury or accompanied by other extra-cranial injuries.

Conclusion

This study found that there was a significant relationship between NSE levels and GCS in traumatic brain injury, namely that the higher the NSE level, the lower the GCS. The Marshall classification in this study has a significant relationship with the NSE level, namely the higher the NSE level, the higher the Marshall classification. It is hoped that in further research, serial evaluations will be carried out to see trends in NSE values before and after surgery. Multivariate analysis needs to be carried out to see the relationship between various factors and NSE levels, such as comorbid data, type of head trauma, and number of traumas.

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Authors' Contributions

All authors contributed to data analysis, article preparation, and paper revision and have collectively assumed responbility for all aspects of this work.

Conflict of Interests

The authors have stated their absence of any conflicts of interest regarding this study.

Ethics Clearance Statement

This study was approved by the Ethics Committee of Dr. Soetomo Surabaya Hospital with no. 0684/KEPK/VI/2023 which was issued in July 2023.

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