Submitted: 20 March, 2024 Accepted: 13 June, 2024 Published: 08 October, 2024 DOI:10.22514/sv.2024.131

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ORIGINAL RESEARCH

Diagnostic role of serum testican and ubiquitin levels in patients with head trauma

Ramiz Yazıcı[1,*], Ayse Fethiye Basa Kalafat[1], Hüseyin Mutlu[2], Eyüp Sarı[3], Utku Murat Kalafat[1], Dilek Atik[4], Başar Cander[5]

[1]Department of Emergency Medicine, Istanbul Kanuni Sultan Suleyman Education and Research Hospital, Health Science University, 34303 Istanbul, Turkey
[2]Department of Emergency Medicine, Aksaray Education and Research Hospital, Aksaray University, 68200 Aksaray, Turkey
[3]Department of Pediatrics, Gulhane Faculty of Medicine, Health Science University, 06018 Ankara, Turkey
[4]Department of Emergency Medicine, Karamanoğlu Mehmetbey University, 70100 Karaman, Turkey
[5]Department of Emergency Medicine, Bezmialem Vakıf University, 34093 Istanbul, Turkey

***Correspondence**
ramiz.yazici@sbu.edu.tr
(Ramiz Yazıcı)

Abstract

We aimed to determine if testican-1 and ubiquitin can serve as early indicators for diagnosing worsening clinical course (presence of intraparenchymal pathology) and mortality in patients with moderate traumatic brain injury (TBI).In this study, we conducted an observational and prospective study in the Emergency Department (ED) of a tertiary care hospital. The department admitted an average of 25,000 patients per month between October 2020 and March 2021. We focused on patients over 18 years old with moderate traumatic brain injury (Glasgow coma score (GCS): 9–13). We compared the prognostic values of blood testican and ubiquitin levels with Receiver Operating Characteristic (ROC) analysis for intracranial pathologies resulting from head trauma. Additionally, we used logistic regression analysis to compare the effectiveness of these markers in diagnosing intraparenchymal injury. The study included a total of 89 patients, with 45 in the case group (moderate TBI) and 44 in the healthy control group. It was found that levels of ubiquitin and testican levels were significantly higher in patients with intraparenchymal pathology ($p = 0.048$, $p = 0.046$, respectively). The cut-off point for detecting intraparenchymal pathology was 161.3 pg/mL for testican (Area under curve (AUC): 0.810; min: 0.654; max: 0.965, $p = 0.002$) and 44.42 ng/mL for Ubiquitin (AUC: 0.863; min: 0.727; max: 1.000, $p < 0.001$). High testican-1 and ubiquitin levels were independent markers for intraparenchymal pathology in moderate head trauma.

Keywords

Biomarkers; Diagnostics; Moderate traumatic brain injury; Testican; Ubiquitin

1. Introduction

Head trauma is a significant health concern that leads to death and disability, requiring long-term treatment and care [1]. Patients with moderate-to-severe TBI makeup about 10% of all TBI cases admitted to hospitals. Many of these patients are admitted to the intensive care unit and receive acute inpatient interventions. The most severe injuries cause the highest burden of death and disability for individual patients [1]. The use of computed tomography (CT) in emergency departments (ED) and the implementation of international treatment guidelines have led to a significant decrease in acute mortality. However, there has been no further improvement in TBI outcomes or acute mortality over the past two decades [2]. While there are scoring systems (GCS, Injury Severity Score (ISS)) and imaging techniques that can reliably predict functional outcomes in severe TBI patients, they still do not adequately to improve the clinical management of severe TBI [3]. There is a need to assess new biomarkers to enhance clinical management and facilitate early diagnosis of severe TBI [3].

Ubiquitin plays a crucial role in identifying normal and abnormal proteins damaged by oxidation in neurons and facilitating their metabolism. It also aids in removing these proteins from neurons. Increased levels of ubiquitin are observed in instances of cell death due to traumatic brain injury, as well as in neurodegenerative diseases such as Alzheimer's and Parkinson's, ischemic or hemorrhagic strokes, neurotrauma, and neurodegeneration conditions like motor neuron diseases. Prior research has demonstrated a marked elevation of ubiquitin levels in individuals with head trauma compared to those without, particularly in cases of blood type (BT) positivity as opposed to BT negativity. Besides its diagnostic function, ubiquitin has also been found to be useful in predicting mortality and monitoring cognitive status [4–6].

Testican-1 is an extracellular matrix proteoglycan found in humans. It has been identified as a cysteine protease inhibitor. Testican-1 is highly expressed, with increased mRNA levels mostly in neurons in the thalamus, hippocampus, and occipital lobe [3]. In animal studies, it has been reported that testican-1, expressed in reactive astrocytes formed after central nervous system (CNS) damage, plays a key role in the successful restoration of brain functions by affecting axonal regeneration [7]. Given this information, both testican-1 and ubiquitin are likely to contribute to improved clinical management, early

diagnosis, and prognosis prediction for patients with TBI. Therefore, this study aimed to determine whether testican-1 and ubiquitin are good indicators of early diagnosis of worsening clinical course (presence of intraparenchymal pathology) and mortality in patients with moderate TBI.

2. Materials and methods

2.1 Study design and setting

This observational and prospective study took place in the Emergency Department (ED) of Kanuni Sultan Suleyman Education and Research Hospital, a tertiary care hospital. The study covered the period between October 2020 and March 2021, during which an average of 25,000 patients were admitted per month. All trauma patients who presented to the ED received treatment according to Advanced Trauma Life Support (ATLS), and informed consent was obtained from the patients' closest relatives. Various data, including demographic characteristics, medical history, vital signs, laboratory findings, trauma mechanism, brain CT findings, length of stay, and mortality status of the patients, were recorded. Trauma scores such as the Glasgow Coma Score (GCS), Injury Severity Score (ISS), and Abbreviated Injury Scale (AIS) were used to assess the severity of the patients' clinical condition. Patients with head trauma were categorized into three groups based on their GCS score: severe (3–8), moderate (9–13), and mild (14–15). The study specifically included patients over 18 years of age with moderate TBI (GCS: 9–13) who provided informed consent. Patients who were under 18 years of age, had missing data, were pregnant, had neurodegenerative disease, had Central Nervous System (CNS) infection, had cerebral palsy, or had penetrating head injury (by a cutting or piercing tool such as a firearm, knife, axe), as well as patients with severe (GCS: 3–8) or mild (GCS: 14–15) TBI, were not included in the study. The control group consisted of healthy individuals without any disease who voluntarily participated in the study. Patients with moderate TBI were divided into two groups: those with intracranial pathology (Subdural hemorrhage, epidural hemorrhage, subarachnoid hemorrhage, intraparenchymal hemorrhage, and contusion) and those without. Treatment was initiated according to ATLS in patients with moderate TBI, and blood samples taken at the time of admission to the emergency department were placed in Ethylenediaminetetraacetic acid (EDTA) tubes. After centrifugation at 2800 rpm for 20 min at 4 °C, plasma samples were placed in Eppendorf tubes and stored at −80 °C until analysis. Levels of testican-1 and ubiquitin were measured in duplicate assays using an enzyme-linked immunosorbent assay (MyBioSource MBS044526; MyBioSource, Inc., San Diego, California). Testican-1 levels were expressed in picograms (pg) per mL, while ubiquitin levels were expressed in nanograms (ng) per mL.

The relationship between the patient's clinical scoring (GCS, ISS and AIS), brain CT results, and mortality status with testican-1 and ubiquitin levels was compared.

2.2 Statistical analysis

All data were analyzed using IBM SPSS Statistics for Windows version 24.0 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp., USA). The Chi-square test was used to evaluate categorical data, and the results were presented as frequencies and percentages. Numerical data were expressed as mean and ±standard deviation. The normality of continuous variables was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests, which indicated that continuous variables did not follow a normal distribution. Therefore, the Kruskal-Wallis test and Mann-Whitney U test were used for the analysis of continuous variables based on the number of groups. Regarding intracranial pathologies resulting from head trauma, blood values of testican and ubiquitin were assessed using the ROC curve (Receiver-Operating Characteristics Curve). The effectiveness of the markers in diagnosing intraparenchymal injury was compared using logistic regression analysis. The results were evaluated for a significance level of $p < 0.05$. G*Power 3.1 software (Allgemeine Psychologie und Arbeitspsychologie, Heinrich-Heine Universitsat, Dusseldorf, Germany) was used for the power analysis of the study. In this observational study, it was determined that a total of 92 patients would be sufficient, assuming an alpha error of 5%, a working power of 80%, and allowing for a 20% loss, based on similar previous studies.

3. Results

In this study, a total of 89 patients were included, with 45 in the case group (with moderate TBI) and 44 in the healthy control group. Within the case group, 62.7% (n = 32) were male, while the control group had 43.2% (n = 19) male participants. The average age of the patients was 29.7 ± 27 years, whereas the control group's average age was 33.8 ± 16.1 years. Upon admission, 84% (n = 38) of the case group had a GCS of 13, 6.7% (n = 3) had a GCS of 12, 2.2% (n = 1) had a GCS of 10, and 6.7% (n = 3) had a GCS of 9. The demographic characteristics of the case and control groups are presented in Table 1.

The levels of both ubiquitin and testican were found to be higher in moderate TBI patients with isolated head trauma compared to patients with multiple trauma ($p = 0.044$, $p = 0.028$, respectively). However, there was no statistical difference in terms of GCS score ($p = 0.462$). The relationship between different parameters and markers in TBI patients is presented in Table 2.

We found that both ubiquitin and testican levels were significantly higher in patients with intraparenchymal pathology ($p = 0.048$, $p = 0.046$, respectively). The effective parameters for detecting intraparenchymal pathology are presented in Table 3. Statistically significant parameters for detecting intraparenchymal pathology were included in the regression model. Variables that were significant in the univariate logistic regression analysis were also included in the multivariate logistic regression model. In the multivariate logistic regression analysis, we found that ubiquitin and testican levels were independent predictors of detecting intraparenchymal pathology

TABLE 1. Clinical and demographic data describing the studied population sample.

Variables	Control group (n: 44) (%)	Patients group (n: 45) (%)	*p* value
Age (yr)	33.8 ± 16.1	29.7 ± 27.0	0.070
Male sex	32 (62.7)	19 (43.2)	<0.001*
Testican (pg/mL)	115.30 ± 69.00	499.88 ± 782.00	0.033
Ubiquitin (ng/mL)	57.3 ± 18.54	127.0 ± 165.30	0.032
GCS (at the time of admission)			
GCS: 13		38 (84.0)	
GCS: 12		3 (6.7)	
GCS: 11		0	
GCS: 10		1 (2.2)	
GCS: 9		3 (6.7)	
Trauma mechanism			
Traffic accident		14 (31.1)	
Fall from height		29 (64.4)	
Assault		2 (4.5)	
Clinical Status			
Discharged as Recovered		23 (51.1)	
Hospitalized in wards		10 (22.2)	
Transferred to the ICU		11 (24.4)	
Exitus		1 (2.3)	
Presence of intracranial pathology		27 (60.0)	

*Presented values are expressed as mean value ± SD. Independent sample t-test, *Pearson Chi-Squared test. GCS: Glasgow Coma Scale; ICU: Intensive Care Unit.*

TABLE 2. Relationship of ubiquitin and testican with different parameters in TBI patients.

Parameters	Ubiquitin (ng/mL)	*p* value	Testican (pg/mL)	*p* value
Those with Isolated Head Trauma	162.9 ± 212.0	0.044*	759.4 ± 965.8	0.028*
Those with Multi Trauma	108.6 ± 138.3		365.6 ± 646.6	
Outcomes				
Discharged	106.1 ± 73.8	0.045¶	422.8 ± 766.0	0.047¶
Hospitalized in wards	124.6 ± 184.1		458.4 ± 326.9	
Transferred to the ICU	150.30 ± 205.7		713.20 ± 1078.00	
Exitus	174.08		765.60	
Glasgow Coma Scale (GCS)				
GCS 13	122.9 ± 175.1	0.319¶	437.6 ± 764.7	0.462¶
GCS 12	270.26 ± 130.1		195.30 ± 504.1	
GCS 10	119.4 ± 125.2		447.0 ± 579.8	
GCS 9	89.1 ± 73.8		334.1 ± 317.4	

*Presented values are expressed as mean value ± SD, ¶Kruskal Wallis test; *Mann-Whitney U test. ICU: Intensive Care Unit.*

(Table 4).

We performed ROC analysis to assess the effectiveness of ubiquitin and testican levels in identifying intraparenchymal pathology (Figs. 1,2). The cut-off point for detecting intraparenchymal pathology was found to be 161.3 pg/mL for testican (AUC: 0.810; min: 0.654; max: 0.965, $p = 0.002$) and 44.42 ng/mL for Ubiquitin (AUC: 0.863; min: 0.727; max: 1.000, $p < 0.001$ as indicated in Table 5.

4. Discussion

After head trauma, some neurons sustain irreversible damage due to mechanical injury in the brain. Neuronal deaths may continue for hours post-injury, and the cascade of cellular,

TABLE 3. Evaluation of parameters related to detecting intraparenchymal pathology.

Parameters	Intraparenchymal pathology Exists (n: 27)	Intraparenchymal pathology Doesn't exist (n: 18)	*p* value
Ubiquitin (ng/mL)	151.1 ± 144.0	121.1 ± 196.5	0.048*
Testican (pg/mL)	598.1 ± 762.4	370.5 ± 709.3	0.046*
Glasgow Coma Scale (GCS)			
GCS 13	22	16	<0.041¶
GCS 12	1	2	
GCS 10	1	0	
GCS 9	3	0	
ISS: Injury Severity Score	19.49 ± 14.18	12.19 ± 10.58	<0.001*
Lactate (mmol/L)	4.3 (3.9–9.1)	3.1 (2.9–7.1)	0.047*

¶*Kruskal Wallis test; *Mann-Whitney U test.*

TABLE 4. Predictors of Intraparenchymal pathology (+) as determined by univariate and multivariate logistic regression analyzes.

Variables	Univariate logistic regression		Multivariate logistic regression	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Ubiquitin (ng/mL)	0.92 (0.85–1.16)	**0.035**	0.98 (0.87–1.24)	<**0.001**
Testican (pg/mL)	0.94 (0.88–1.38)	**0.044**	0.96 (0.84–1.44)	**0.002**
Glasgow Coma Scale (GCS)	0.85 (0.76–1.31)	**0.041**	0.98 (0.88–1.76)	0.097
ISS: Injury Severity Score	1.02 (0.96–1.51)	<**0.001**	1.11 (0.98–1.96)	**0.043**

OR: odds ratio; CI: confidence interval. The p-values that are significant are written in bold.

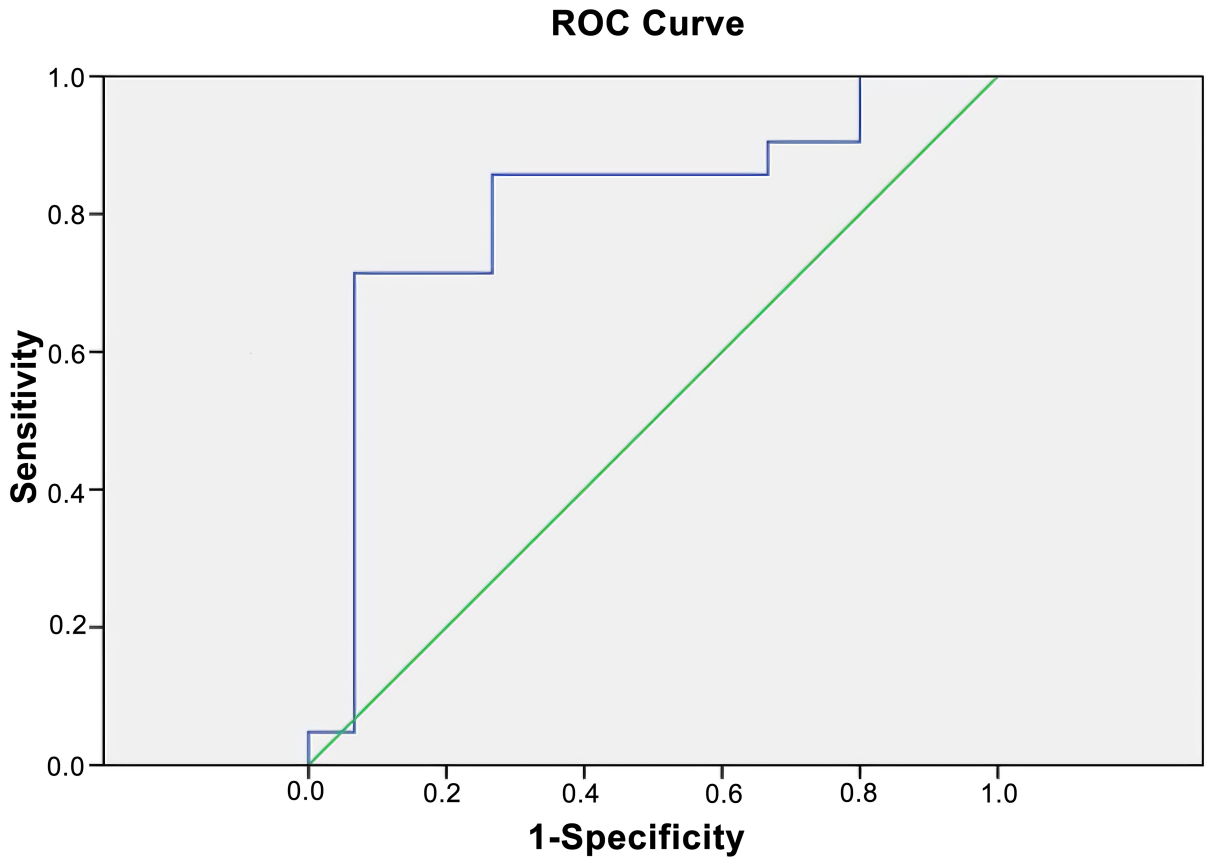

FIGURE 1. Receiver-operating characteristic curves of Ubiquitin to detect intraparenchymal pathology. ROC: receiver operating characteristic.

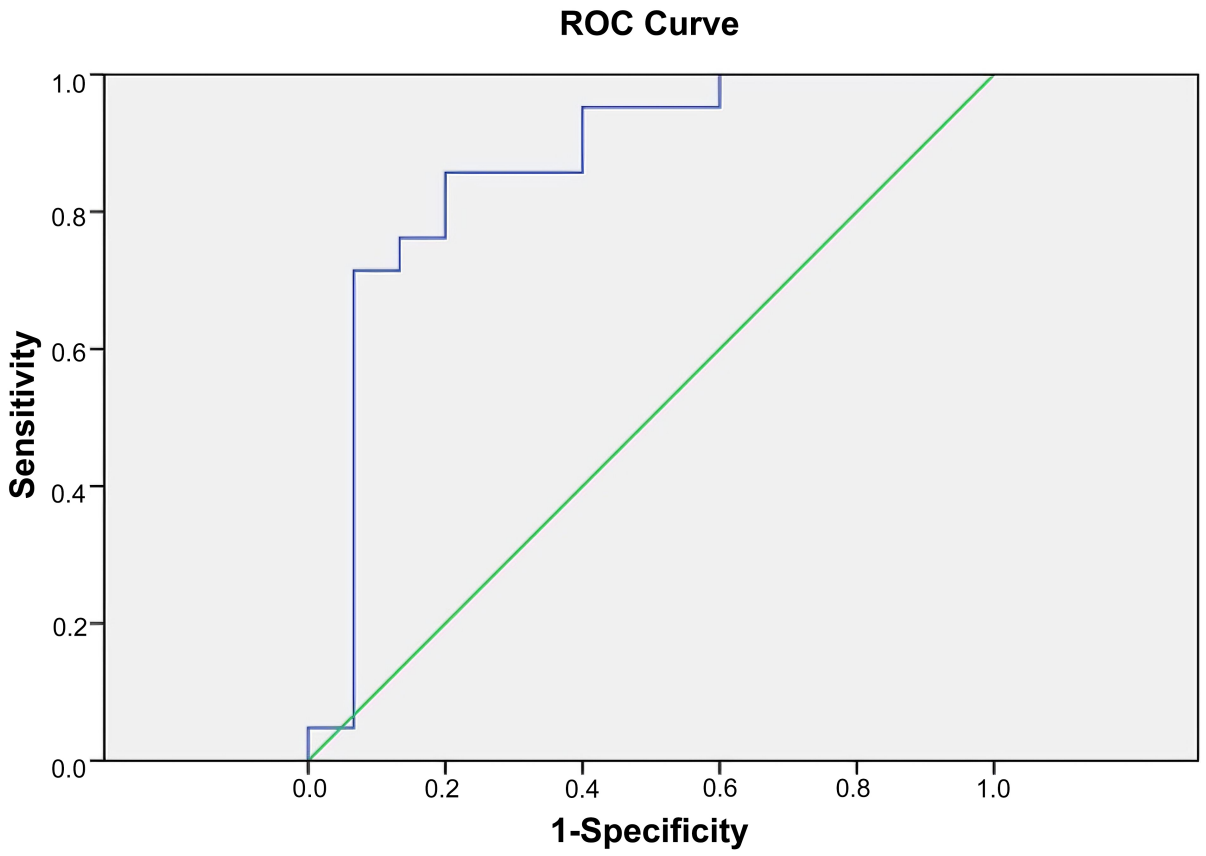

FIGURE 2. Receiver-operating characteristic curves of Testican to detect intraparenchymal pathology. ROC: receiver operating characteristic.

TABLE 5. ROC curve analysis for the prediction of intraparenchymal pathology (+).

Variables	AUC (95% CI)	Cut-off value	Sensitivity %	Specificity %	+LR	−LR	PPV (%)	NPV (%)	*p* value
Ubiquitin (ng/mL)	0.863 (0.727–1.000)	>44.42	86.14	95.47	2.04	0.74	95.0	54.3	0.016
Testican (pg/mL)	0.810 (0.654–0.965)	>161.3	88.42	88.61	2.16	0.59	91.3	46.4	<0.001

ROC: receiver operating characteristic; AUC: area under the ROC curve; CI: confidence interval; +LR: positive likelihood ratio; −LR: negative likelihood ratio; PPV: positive predictive value; NPV: negative predictive value.

molecular, and biochemical processes determines these deaths [1, 8]. The most crucial of these cascades is the local inflammatory response in the damaged brain tissue [8, 9]. Research studies have shown that after an injury, reactive astrocytes express a variety of biochemical markers, which are linked to local inflammatory responses. These markers have been found to promote axonal regeneration, indicating their crucial role in assisting neural repair processes. The interaction between reactive astrocytes and these biochemical markers not only helps reduce neural tissue damage but also actively contributes to regenerative cascades, emphasizing their importance in understanding post-injury neural recovery mechanisms [7–10]. In patients with head trauma, using these markers and scoring methods may help detect intracranial pathology and predict mortality. Our study is the first to assess the effectiveness of testican-1 and ubiquitin in identifying intraparenchymal pathology in patients with moderate TBI.

In our study, we observed significantly higher levels of testican-1 and ubiquitin in patients with moderate TBI compared to the control group ($p = 0.032$, $p = 0.033$). We found that testican-1 exhibited 88.42% sensitivity and 88.61% specificity (AUC = 0.810, 95% CI: 0.654–0.965) at a cut-off value of 161.3 for predicting intraparenchymal pathology. Additionally, we observed that ubiquitin demonstrated 86.14% sensitivity and 95.47% specificity at the cut-off value of 44.42 (AUC = 0.863, 95% CI: 0.727–1.000). These results suggest that both testican-1 and ubiquitin could serve as effective markers for predicting intraparenchymal pathology in head trauma patients.

In a separate animal study, Liu and colleagues noted an increase in ubiquitin levels in Cerebrospinal fluid (CSF) and serum of rats with acute TBI [11]. Another study by Welch *et al.* [12], which included 251 patients with moderate and mild TBI, found that ubiquitin had 100% sensitivity and 39% specificity at a cut-off value of >40 pg/mL in detecting intracranial pathology. It's important to note that this partic-

ular study exclusively recruited patients with head trauma and did not include a control group of healthy volunteers, which differs from our investigation. This difference may have contributed to variations in our sensitivity and specificity values. Furthermore, a study by Papa *et al.* [13] reported an increase in ubiquitin levels within an hour after injury in 86 TBI patients, and these levels were correlated with the GCS score, proving effective in detecting intracranial pathology. Similarly, Mondello *et al.* [14] also reported that ubiquitin was effective in detecting intracranial pathology and was associated with mortality in pediatric patients with head trauma. In our study, our findings for ubiquitin were consistent with the literature. However, we did not observe a correlation between ubiquitin levels and GCS scores.

In an animal study, Iseki *et al.* [7] found that testican-1 was expressed in large amounts in reactive astrocytes in the mouse brain following head trauma. Shultz *et al.* [3] discovered that testican-1 levels increased in the CSF of 16 patients with severe TBI. Our study revealed that the level of testican-1 in patients with moderate TBI was significantly higher than in the control group ($p = 0.032$). We also found that a testican-1 level above 161.3 pg/mL displayed high sensitivity and specificity (88.42% and 88.61% respectively). These results suggest that both testican-1 and ubiquitin levels were elevated in patients with head trauma, and these markers showed significant potential in identifying intraparenchymal pathology with notable sensitivity and specificity.

However, our study had some limitations. Firstly, it was conducted in a single center with a relatively small sample size, so the results need to be verified with larger case data. Secondly, we only measured testican-1 and ubiquitin levels once and did not take serial measurements. We also only evaluated plasma levels, without simultaneously assessing levels in the CSF. Further investigation is needed to understand the role of excitoxicity, ischemia, and hypoperfusion on biomarkers associated with cell death in traumatic brain injury. Lastly, due to the limited number of patients, we did not study the relationship between CT findings and biomarker levels.

5. Conclusions

We discovered that elevated levels of testican-1 (<161.3) and ubiquitin (<44.42) serve as independent markers for intraparenchymal pathology in cases of moderate head trauma. If a patient arrives at the emergency department with a head injury and has a testican-1 level exceeding 161.3 or a ubiquitin level surpassing 44.42, it is imperative to closely monitor the patient and commence early intervention. It is essential to conduct prospective studies with larger sample sizes to validate these findings.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article.

AUTHOR CONTRIBUTIONS

RY, AFBK, UMK, DA and BC—were responsible for the study concept and study design; critical revision of the manuscript for important intellectual content. RY and AFBK—performed data extraction. ES and HM—were responsible for data analysis. RY, UMK, DA, BC—drafting of the manuscript. All authors have read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethics committee of Kanuni Sultan Suleyman Training and Research Hospital with the approval number 2019/159, and was conducted under the guidelines outlined in The Declaration of Helsinki on Medical Research involving Human Subjects. Informed consent was obtained from all individual participants involved in the study. The study was registered in the ClinicalTrials.gov (NCT06537713).

ACKNOWLEDGMENT

Not applicable.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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How to cite this article: Ramiz Yazıcı, Ayse Fethiye Basa Kalafat, Hüseyin Mutlu, Eyüp Sarı, Utku Murat Kalafat, Dilek Atik, *et al*. Diagnostic role of serum testican and ubiquitin levels in patients with head trauma. Signa Vitae. 2024; 20(10): 106-112. doi: 10.22514/sv.2024.131.