Surveying the changes in serum ubiquitin C-terminal hydrolase-L1 levels in severe traumatic brain injury patients

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Summary

Objective: To investigate the change in serum ubiquitin C-terminal hydrolase-L1 protein (UCH-L1) levels in severe traumatic brain injury patients. *Subject and method:* Thirty-nine patients with severe traumatic brain injury were treated at the surgical critical care department, 103 Military Hospital. The patients were treated to the protocol, and information was collected in the medical records for research and testing. UCH-L1 at time T_0, T_1, T_2, T_3, T_4 and T_5. A prospective cohort study. *Result:* Serum UCH-L1 concentration gradually increased from T_0 (9.18ng/mL) to T_1 (11.59ng/mL) and peaked at T_2 (19.9ng/mL), then gradually decreased over time. The median serum UCH-L1 concentration in the surviving group was constantly lower than in the death group. The biggest difference observed at T_1 (10.47ng/mL and 46.76ng/mL), however, was not statistically significant with p=0.063, while the difference was statistically significant at T_3, T_4, and T_5. *Conclusion*: Serum UCH-L1 levels in patients with severe TBI increased gradually from T_0 to T_1 and peaked at T_2, then gradually decreased at T_5. Serum UCH-L1 levels in the surviving group were constantly lower than those in the death group, but there was no significant difference between T_0, T_1, and T_2, whereas at points between T3, T4, and T5, the difference was statistically significant.

Keywords: Traumatic brain injury, biomarker.

1. Background

Accurate evaluation of brain damage in traumatic brain injury (TBI) patients is critical for neurological prognosis as well as maintaining the risks and benefits of therapy. The prognosis of outcome remains difficult since neurological examination is frequently impacted by the use of sedatives, analgesics, and muscle relaxants. The foregoing parameters have no effect on neuroimaging; nonetheless, it has numerous limitations, including inaccuracy in patients with diffuse axonal injury, increased intracranial pressure in the presence of mass lesions, and inability to effectively use predictive information [1]. Furthermore, computed tomography did not evaluate the lesions at the molecular, cellular, and metabolic levels, as well as the risk of secondary injury. On the other hand, biomarkers are simple to quantify and can indicate the severity of brain injury and neuropathological processes. Ubiquitin C-terminal hydrolase-L1 protein (UCH-L1) is abundant and specific in neuronal cells. It is involved in increased or transferred ubiquitin from aberrant proteins, including eccentric proteins, and damaged proteins due to oxidation or degradation. Many investigations have indicated the elevation in UCH-L1 levels in the

***Received**: 5 September 2022, **Accepted**: 4 January 2023*
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cerebrospinal fluid and serum in severe TBI patients. However, in Vietnam, there have been no studies investigating the changes in UCH-L1 levels in TBI patients. Therefore, we conducted a study with the following objective: To investigate the change in serum UCH-L1 levels in severe TBI patients.

2. Subject and method

2.1. Subject

Studied on 39 patients diagnosed with severe traumatic brain injury treated at the Surgical Critical Care Department, Center of Emergency Critical Care Medicine and Clinical Toxicology, 103 Military Hospital from January 2021 to March 2022.

2.1.1. Inclusion criteria

Patients aged 16 years and over.

Patients were diagnosed with isolated severe traumatic brain injury (Glasgow admission score from 3 to 8 points).

Hospitalization within 6 hours of injury.

Family or legal representative consented to participate in the study.

2.1.2. Exclusion criteria

The patient was diagnosed with ischemic or subarachnoid bleeding or traumatic brain injury or the patient who experienced brain surgery one month before.

Having neurodegenerative diseases such as Alzheimer's, Parkinson's, mental disorders, and brain atrophy in old age.

Traumatic brain injury in the setting of multiple trauma. The patient was not hemodynamically maintained after initial resuscitation (systolic blood pressure < 90mmHg).

2.2. Method

2.2.1. Research design and sample collection

Research design: Prospective descriptive study

Sample collection method: Convenient sampling collection.

2.2.2. Study timelines

T_0: Time of admission in the Surgical Critical Care Department.

T_1: 6 hours after admission.

T_2: 12 hours after admission.

T_3: 24 hours after admission.

T_4: 48 hours after admission.

T_5: 72 hours after admission.

2.2.3. Research contents

Age: Divided by age groups: 20, 21-40, 41-60, > 60.

Gender: Survey the ratio of men and women.

Causes of TBI: Traffic accidents, falls, the other causes.

Death in 28 days.

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) test:

The blood-brain barrier transports proteins from the brain into the bloodstream. When the integrity of this barrier is damaged in the case of TBI, the ability to transport these proteins into the bloodstream can be increased. Therefore, when TBI happens, it will increase the number of some biomarkers in the peripheral blood, and each different location of the nervous system will have its own biomarkers.

UCH-L1 is the abundant protein in the brain; notably, it is estimated that UCH-L1 makes up about 1-5% of the total protein in neurons. UCH-L1 maintains an intracellular reservoir of ubiquitin, which is essential in maintaining the balance of the proteolytic function. UCH-L1, which can regulate the ubiquitin pool involved in the mitochondrial exchange, is also a part of the cell survival response, or it has a significant role in disease progression [2].

Each time for testing, 2mL of the patient's venous blood was retrieved and sent to the hematology department within 30 minutes.

Blood samples were centrifuged in a test tube at 1000rpm for 15 minutes at 40°C, then separated for serum.

If serum was not used for testing immediately, it would be stored at -80°C in a deep freezer. The shelf life of samples was less than three months.

Serum samples were used for testing using the ELISA method with the MyBioSource KIT, then a microtitration spectrometer was utilized to read the results [3].

Machine reads ELISA results UCH-L1 at Department of Pathophysiology - Viet Nam Military Medical University.

UCH-L1 concentration in healthy people 0.02-0.13ng/mL.

Investigation of changes in serum UCH-L1 levels at time T_0, T_1, T_2, T_3, T_4, T_5.

2.2.4. Research steps

Patients with severe TBI admitted to the Surgical ICU - 103 Military Hospital for emergency and treatment were included in the study. The patients were treated follow to the intensive care regimen for severe TBI of the surgical critical care department - 103 Military Hospital.

Determine age, sex, and cause of TBI according to medical records.

Test serum UCH-L1 levels at time T_0, T_1, T_2, T_3, T_4, T_5.

Determination of death in 28 days.

3. Result

3.1. General characteristics

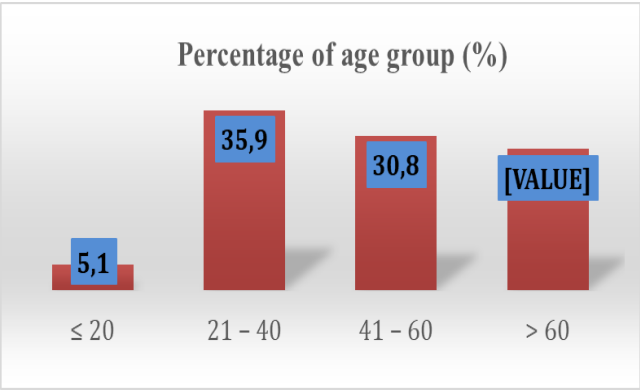

Figure 1. The distribution in age group of the patients involved in the study.

Comment: Most of the patients in the study were 21-40 years old (35.9%).

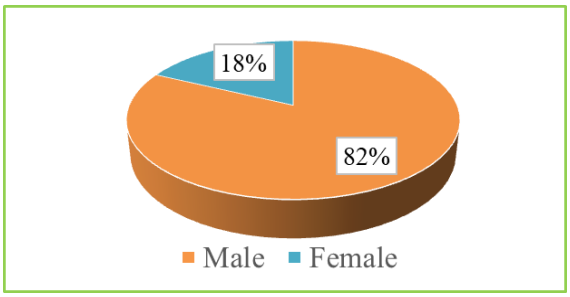

Figure 2. The sex ratio of the patients involved in the study.

Comment: Men accounted for a high percentage (82.1%) of the study patients.

Table 1. TBI Causes

Causes \ Value	Patients (n)	Proportion (%)
Traffic accident	25	64.1
Falls	11	28.2
Another Causes	3	7.7
All	39	100

Comment: The main cause of TBI was traffic accidents (64.1%), followed by falls (28.2%).

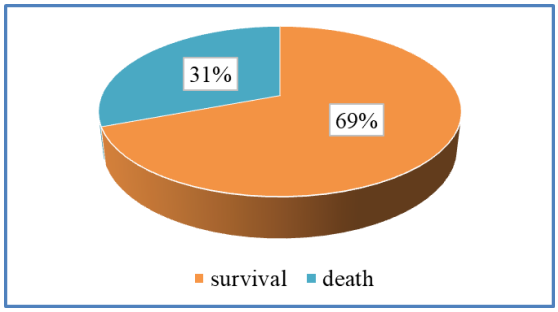

Figure 3. The mortality rate due to sTBI of the patients involved in the study.

Comment: The mortality rate due to severe TBI accounted for 30.8%.

3.2. Changes in serum UCH-L1 levels

Table 2. Changes in serum UCH-L1 levels at the times

Points	Median value (ng/mL)
T_0	9.18 (2.74-16.79)
T_1	11.59 (4.94-46.76)*
T_2	19.9 (8.66-33.41)*
T_3	10.95 (5.78-28.49)*
T_4	9.02 (5.70-20.82)
T_5	9.11 (4.71-16.43)

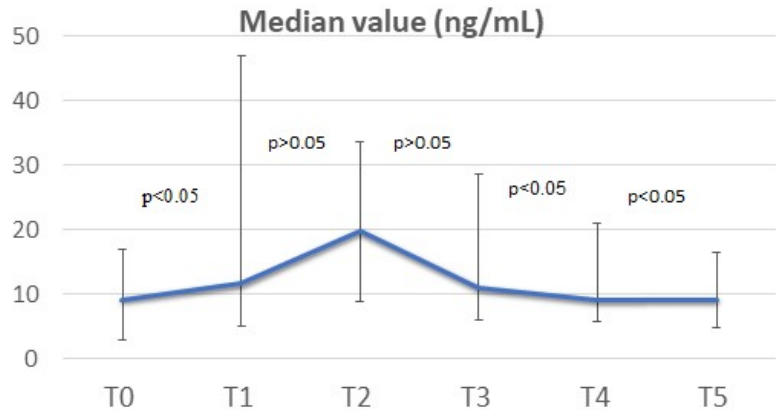

Figure 4. Median changes in serum UCH-L1 levels against time post-admission.

Comment: Serum UCH-L1 concentration moderately increased from T_0 (9.18) to T_1 (11.59) and peaked at T_2 (19.9), then gradually decreased over time.

Table 3. Changes in serum UCH-L1 levels by age

Time (n_1, n_2)	Median Value (ng/mL)		p
	age ≤ 60	age > 60	
T_0 (27, 12)	7.2 (2.1-14.9)	11.3 (7.4-38.3)	0.059
T_1 (27, 12)	10.5 (2.4-44.6)*	20.3 (9.5-49.7)*	0.181
T_2 (27, 12)	17.5 (6.2- 33.4)*	22.6 (12.2-41.2)*	0.235
T_3 (27, 12)	8.5 (4.2- 22.5)	25.6 (13.1-41.3)*	0.025
T_4 (27, 12)	6.6 (3.9-13.3)	16.8 (11.5-29.5)	0.006
T_5 (27, 12)	5.8 (3.8- 11.3)	13.3 (9.7-22.5)	0.012

*Note: n1: Number of patients group ≤ 60 years old. n2: Number of patients group > 60 years old. *: Difference value from T0 with p<0.05.*

Comment: UCH-L1 concentration in the group over 60 years old was significantly higher than that in the group under 60 years old at the time T_3 to T_5 (p<0.05, Mann-Whitney test).

Table 4. Changes in serum UCH-L1 levels between the living and dead groups

Time (n_1;n_2)	Median value (ng/mL)		p
	Living groups	Dead group	
T_0 (27, 12)	8.65 (2.44-13.08)	15.07 (2.65-55.76)	0.128
T_1 (27, 12)	10.47 (2.31-20.47)*	46.76 (4.94-69.10)*	0.063
T_2 (27, 12)	16.13 (8.02-28.37)*	23.02 (8.66-69.57)*	0.136
T_3 (27, 12)	9.30 (5.57-21.76)	31.07 (11.17-67.72)	0.007
T_4 (27, 12)	8.52 (4.55-11.56)	23.15 (9.16-48.41)	0.008
T_5 (27, 12)	6.22 (4.08-10.90)	17.86 (7.27-50.77)	0.012

*Note: n1: Number of patients in the living group. n2: Number of patients in the dead group. *: Difference value from T0 with p<0.05.*

Comment: The median serum UCH-L1 concentration in the living group was constantly lower than that in the dead group. In comparision with T0, the biggest difference of UCH-L1 level was observed at T1 (10.47ng/mL and 46.76ng/mL), however, this was not statistically significant with p=0.063, while the difference was statistically significant at the time of T_3, T_4, T_5.

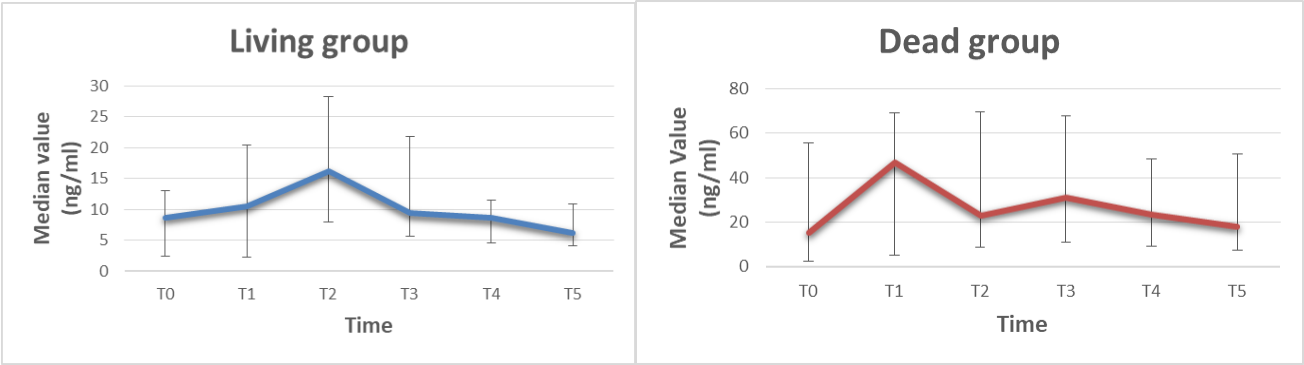

Figure 4. Serum UCH-L1 levels in the living and dead groups.

Comment:

Living group: The median of serum UCH-L1 concentration at T_0 (8.65ng/mL) gradually increased at T_1 (10.47ng/mL), peaked at T_2 (16.13ng/mL), then decreased and was the lowest at T_5.

Death group: The median of serum UCH-L1 concentration at T_0 (15.07ng/mL) rose and peaked at T_1 (46.76ng/mL), then dropped throughout time and was the lowest at T_5.

4. Discussion

4.1. General characteristic

Nguyen Thanh Hai conveyed a study 188 TBI patients at Viet Duc Hospital and found that 12.5% of subjects were severe, where 75% of patients aged 21-60, 77.1% were male, and 22.9% were female [4]. In our study, men accounted for 82%, and most of them were of working age. The majority of study population were in the age group of 21-40 years. This result is consistent with our study. Thus, most patients with severe TBI were male and young, possibly due to job characteristics, the proportion of men who drink alcohol and stimulants while participating in traffic a lot, and the lack of compliance with safety laws. Unsafe traffic (do not wear a helmet, over-speed) is more common in men than in women which can be linked to the proportion of men in the studies is higher than in women.

According to our research, the main cause of severe traumatic brain injury is traffic accidents (64.1%), then falls (28.2%), and the other causes are less common (beating, being hit in the head by a fallen tree, sports accidents, etc.). This result is consistent with the previous research of many authors, such as the study conveyed by Nguyen Dinh Hung in 2018, in which the cause of traffic accidents accounting for 77.27%, followed by daily-life accidents (19.7%) [5].

The mortality rate of severe TBI patients in the intensive care unit-of Alexandria University Hospital of Taysser Zaytoun in 2017 was 18.3% [6]. This rate is higher in our study. The difference can be accounted due to the defference in studied patients as Zaytoun only took patients with a Glasgow score of 5-7, while our patient was more severe, with a Glasgow score of 4. Furthermore, we had 28.2% of patients over 60 years old, while the patients in Zaytoun's study ranged from 30-43 years.

4.2. Changes in serum UCH-L1 levels

Serum UCH-L1 levels in patients with severe TBI increased gradually from T_0 to T_1 and peaked at T_2, then gradually decreased to T_5. According to Ruchira, after TBI, angioedema and cytotoxic edema occur from a few hours to a few days, and even the

blood-brain barrier is damaged immediately after TBI, and the damage is more severe due to molecular mechanisms, metabolic disorders, and substances caused inflammation. This may be the cause of the increase of UCH-L1 levels and peak at T_2 time (12 hours after injury) [7]. Linda Papa investigated the kinetics of GFAP and UCH-L1 in 325 patients with mild to moderate TBI. Patients admitted to the hospital were diagnosed with mild to moderate TBI, and blood samples were taken every 4 hours after injury (after injury 4, 8, 12, 16, 20, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180 hours). Authors' drawn conclusions was the UCH-L1 concentrations increased rapidly after injury, peaked at 8 hours, and then gradually decreased [8]. The reason of that such finding was that the participants in Papa' research with mild and moderate TBI, in contrast, we only studied on patients with severe TBI.

Our finding reavealed that serum UCH-L1 levels were higher in the age group 60 and older than in the group under 60 years old, and the difference was significant from the 24th hour after admission. This can be explained by age as a predictor of mortality, according to many studies. The danger of dying increases as one gets older. Older age, poorer compensatory capacity, and traumatic brain injury on the background of elderly patients would be less reversible, leading to higher UCH-L1 levels than younger age groups. According to the hypothesis, the UCH-L1 concentration in the first 12 hours depends mainly on the characters of the lesion, so in the first hours, the serum UCH-L1 concentrations of the two groups did not have much difference. This observation is the foundation for determining the relationship between UCH-L1 concentration and age.

The variation of serum UCH-L1 levels over time in the surviving group was lower, but there was no difference compared with the death group at times T_0, T_1, and T_2. Meanwhile, at the time of T_3, T_4, and T_5, the concentration of UCH-L1 in the death group was statistically significantly higher than that in the surviving group. Ubiquitin levels rise promptly after neuroral injury as a result of a protein breakdown product [2] as UCH-L1 is a de-ubiquitin-reducing protein, so it can be hypothesized that the protein degrades soon after damage. Increased ubiquitin metabolism is followed by a rise in UCH-L1 levels, which peaks about 12 hours after damage when ubiquitin metabolism is halted by some mechanism. After the gradual reduction, ubiquitin concentration decreased, along with the decrease of UCH-L1, starting from the 12th to the 24th hour onwards. This may be because UCH-L1 was released into the extracellular fluid after damage to CNS neurons. The more severe the injury, the more injury the neuronal damage, and the more UCH-L1 was removed. From there, the extracellular fluid penetrates into the network of blood vessels damaged by the mechanism of injury and then enters the general circulation.

In 2012, Mondello S et al examined the biological properties of serum UCH-L1 as a prognostic marker in 95 severe traumatic brain injury patients based on the Glasgow Coma Scale (GCS < 9). The results showed that serum UCH-L1 in both groups was detected very early (< 6 hours after admission) at 5.326 ± 1.1ng/mL and increased sharply in the first 24 hours at 2.096 ± 0.43ng//mL. Peaking at 12 hours is consistent with our study. Serum UCH-L1 levels were markedly increased in the death group compared with the surviving group [9]. However, the author did not make specific comparisons of the time, so there are points that are different from our study design.

In our study, the surviving group had the highest serum UCH-L1 concentration at T_2 and then gradually decreased, while the death group had the highest increase in serum UCH-L1 at the time of T_1; after that, it also reduced. The difference at the peak time of serum UCH-L1 levels may be due to the more severe damage in the death group, the greater degree of neuronal destruction, and the blood-brain barrier, so the UCH-L1 concentration increased earlier. Gretchen M Brophy et al 2011 assessed the kinetics of UCH-L1 in body fluids in patients with severe traumatic brain injury. The authors reported that the peak serum UCH-L1 concentrations in the

death group (1.9ng/mL) were statistically significantly higher than the surviving group (0.23ng/mL) as well as the longer time to reach this level. Peak concentrations in the dead group (9 hours) surged faster than in the living group (13 hours) [10].

5. Conclusion

Serum levels of UCH-L1 in severe TBI patients increased gradually from T_0 to T_1 and peaked at T_2 then moderately decreased to T_5.

Serum concentrations of UCH-L1 in the age group 60 and older were higher than that in the group under 60 years old; the difference was significant from the 24th hour after admission.

Serum levels of UCH-L1 in the surviving group were consistently lower than those in the death group, but there was no difference at the time of T_0, T_1, T_2, while at the time of T_3, T_4, and T_5 there was a statistically significant difference.

The Surviving group had the highest serum UCH-L1 levels at the time of T_2 and then gradually decreased, while the death group had the highest serum UCH-L1 levels at the time of T_1, then also decreased.

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