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Serum concentration of ubiquitin c-terminal hydrolase-L1 in detecting severity of traumatic brain injury

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Abstract. One of the main problems with head injury is assessing the severity. While physical examination and imaging had limitations, neuronal damage markers, ubiquitin C-terminal hydrolase-L1 (UCH-L1), released in the blood may provide valuable information about diagnosis the traumatic brain injury (TBI). Analyzing the concentrations of serum ubiquitin C-terminal hydrolase-L1 (UCH-L1), there must have a neuronal injury biomarker, in the TBI patients serum and their association with clinical characteristics and outcome. There were 80 TBI subjects, and there are mild, moderate, and severe involved in this study of case-control. By using ELISA, we studied the profile of serum UCH-L1 levels for TBI patients. The UCH-L1 serum level of moderate and severe head injury is higher than in mild head injury (p<.001), but we didn’t find a specific difference between moderate and severe head injury patients. There is no particular correlation found between serum UCH-L1 level and outcome. Serum levels of UCH-L1 appear to have potential clinical utility in diagnosing TBI but do not correlate with outcome.

1. Introduction
TBI is an emerging health issue in the world. Besides the high cost of therapy, CDC estimates that at least five million Americans need long-term assistance to perform daily activities related to the sequel of prolonged head injury. The incidence of global TBI has increased in the past two decades, possibly due to increased use of motor vehicles in developing countries. It was difficult in diagnosing and predicting the outcome after acute TBI based on clinical and radiological conditions. Diagnostic modalities are needed to diagnose the severity of TBI and predict the outcome. Protein degradation in secondary brain injury was said to be mediated by ubiquitin, in which ubiquitin C-terminal hydrolase-L1 (UCHL-1) concentrated in neuronal cells. As supposed to be a promising biomarker in neuropathology, this study aimed to determine the association of serum UCHL-1 levels with the severity of TBI.

2. Method
This study is a cross-sectional study conducted in all patients with head injuries aged 18-50 years with the onset of head injury events under 48 hours starting from June 2012 to March 2013. Patients with multiple injuries, surgical indications, pregnancy, other trauma, or with other neurological disorders were from the study. At the beginning of admission, subjects were treated with ATLS principles to stabilize their conditions. Brief history taking in the second survey was made. Moderate and severe head injuries are according to the procedure. Standard laboratory and radiological examinations were
carried out, such as complete blood, blood gas analysis, blood glucose level, blood electrolytes, liver function, kidney function, and blood clotting function. Standard radiological examinations performed were lateral cervical projection X-rays, AP Thorax, Pelvic AP, and Head CT Scan, according to the standard hospital procedure. The patient or patient's family know any additional examination for this study. On the consented subjects, the serum from the blood that has been taken and stored for further laboratory examination.

UCHL1 levels were by measuring ELISA method using Chemwell 2910 (Awareness Technology, Inc.). The reagents used for the examination are the Enzyme-Linked Immunosorbent Assay Kit for Ubiquitin Carboxylic Terminal Hydrolase L1 (UCHL1) Homo Sapiens production USCN Life Science Inc. (Wuhan, China). The minimum detectable dose for UCHL-1 in this reagnsia is less than 0.056 ng / mL with a coefficient of variation (CV) intra-assay<10% and intra assay<12%.

Data were collected and analyzed with SPSS. Categorical variables were regarding frequency and percentage, presented in both tabular and graphics. Descriptive analysis of numerical variables is in the form of the centralized size (mean, median) and the size of the spread (standard deviation, minimums). If the distribution of normal data, use the pair of mean and standard deviation. If the data distribution is abnormal, the median is with minimums. For normality test, Kolmogorov-Smirnov test was used with p <0.05 significance. For analysis of unpaired data with normal distribution, the author used One Way ANOVA test with Bonferroni as post hoc test. If the data distribution is not normal, Kruskal Wallis test is used. Correlation of numerical variables by statistical using Pearson correlation test when there is the distribution of normal data. If the data distribution is not normal, Spearman test is used.

3. Result
A total of 80 subjects followed this study with details of 25 people with mild TBI, 29 people with moderate TBI, and 26 people with severe TBI. Mean age of subjects in this study was 29-32 years, with the predominantly male. The ethnicities were distributed as Mandailing (n = 22), Batak Karo (n = 16), Aceh (n = 16), Batak Toba (n = 13), and Java (n = 8) (Table 1).

| Demographics | Mild head injury (n=25) | Moderate head injury (n=29) | Severe head injury (n=26) |
|--------------|-------------------------|----------------------------|--------------------------|
| Age (years old) | 31.8 ± 11.4 | 32.07 ± 9.13 | 29.4 ± 8.1 |
| Range of age (years old) | 18-54 | 18-49 | 19-44 |
| Gender | Male | 72 | 82.8 | 76.9 |
| | Female | 28 | 17.2 | 23.1 |
| Ethnicity | Batak Toba | 12 | 17.2 | 19.2 |
| | Batak Karo | 20 | 20.7 | 19.2 |
| | Mandailing | 28 | 27.6 | 26.9 |
| | Aceh | 24 | 20.7 | 15.4 |
| | Javanese | 8 | 10.3 | 11.5 |
| | Others | 8 | 3.4 | 7.7 |
| GCS on admission (range) | 14-15 | 9-13 | 5-8 |

There was no significant difference in UCHL-1 levels in male (513.40 ng/mL) and female serum (662.23 ng/mL) with Mann-Whitney test (p=0.712; Table 2). Levels of UCHL-1 in the five major tribes were not significantly different (p=0.590; Table 2).
Table 2. The difference of UCHL-1 level based on genders and ethnicities.

|                | N  | Mean       | Median    | Range       | P     |
|----------------|----|------------|-----------|-------------|-------|
| **Gender**     |    |            |           |             |       |
| Male           | 62 | 513.40     | 399.58    | 12.42-1947.02 | 0.712 |
| Female         | 18 | 662.23     | 322.12    | 6.21-2969.02 |       |
| **Ethnicity**  |    |            |           |             |       |
| Mandailing     | 22 | 488.92     | 423.46    | 23.84-1371.76 | 0.590 |
| Batak Karo     | 16 | 408.08     | 309.89    | 65.97-1084.64 |       |
| Aceh           | 16 | 510.42     | 365.75    | 17.07-1947.02 |       |
| Batak Toba     | 13 | 630.63     | 485.90    | 27.16-1482.71 |       |
| Java           | 8  | 980.75     | 693.45    | 12.42-2969.02 |       |

UCHL-1 serum levels in mild, moderate, and severe TBI groups were 164.97 ± 93.71 ng/mL; 728.12 ± 472.23 ng/mL; and 712.00 ± 567.32 ng/mL, respectively. There was a significant difference of UCHL-1 serum levels between those groups by ANOVA test. Post hoc Bonferroni test showed no significant difference was between moderate and severe TBI groups (Table 3).

Table 3. UCHL-1 serum levels in mild, moderate, and severe TBI.

| Group          | N  | UCHL-1 serum level (ng/mL) | P     |
|----------------|----|---------------------------|-------|
| Mild TBI       | 25 | 164.97 ± 93.71            | 0.0001a|
| Moderate TBI   | 29 | 728.12 ± 472.23           | (a)   |
| Severe TBI     | 26 | 712.00 ± 567.32           | (b)   |

*aOne Way ANOVA

In this study, seven research subjects died within the first three days. Three subjects were those with moderate TBI, and four subjects were those with severe TBI. In the mild head injury group, no subjects died within the first three days after the accident.

UCHL-1 serum levels in the decedent group were seen to be relatively higher than the survival group (989.98 ng/mL versus 504.70 ng/mL), but the difference was not significant (p = 0.712; Table 4). No significant correlation was between the GCS at admission and duration of hospital stay (Table 5).

Table 4. UCHL-1 serum level in the first three days in decendent group.

| Group      | N  | Mean   | Median   | Range       | P     |
|------------|----|--------|----------|-------------|-------|
| Decendent  | 7  | 986.98 | 602.23   | 269.86-2699.18 | 0.712a|
| Survival   | 73 | 504.67 | 413.13   | 6.21-1947.02  |       |

*aMann-Whitney test

Table 5. Correlation of UCHL-1 serum level with GCS at the time of admission and there is the duration of hospital stay.

| Correlation                  | r    | P    |
|-----------------------------|------|------|
| GCS at the time of admission| -0.198 | 0.193 |
| Duration of hospital stay   | 0.123 | 0.138 |

4. Discussion

Advances in laboratory technology and the development of proteomic subdivisions have enabled the discovery and rapid detection of new biomarkers that were previously unavailable. UCHL-1 is a protein that highly expressed in numbers in neurons. Protein degradation in secondary brain injury
was said to be mediated by ubiquitin. Ubiquitin C-terminal hydrolase-L1 (UCHL-1) concentrated in neuronal cells. Previous UCHL-1 activity abnormality disorder correlates with several degenerative neurological disorders, such as Parkinson’s, Huntington’s, and Alzheimer’s. Kobeissy et al. (2006) reported a doubling in UCHL-1 in the first 48 hours after induced TBI in mice. The same results were found by Liu et al. (2010) that UCHL-1 levels increased within the early 48 hours after traumatic injury to brain tissue as well as in the cerebrospinal fluid. They found that UCHL-1 levels would increase in patients with severe head injury compared to controls in the first 168 hours after TBI. The increase of UCHL-1 levels in TBI was first reported by Mondello et al. (2012). They found that UCHL-1 levels would continue to increase even up to seven days after the trauma. In this study, the similar result was found. UCHL-1 is a large-size protein (24 kDa) that should not be able to penetrate the blood-brain barrier. As we know, the blood-brain barrier is an integrated structure that does not allow proteins with molecular weight >500 Dalton to pass. It might penetrate the blood-brain barrier due to damage to blood-brain barrier after trauma. Currently, several polymorphisms of the UCHL-1 gene was in the Swedish population. We found no difference in UCHL-1 levels among the difference of tribes.

In this study, there was no association between level of awareness at admission (GCS) with UCHL-1 level. This result was different from Mondello et al.’s findings (2012) as UCHL-1 levels in severe TBI patients with GCS 3-5 were significantly higher than those with GCS 6-8. There are two possible causes for the absence of such a relationship. First, in this study, serum taking was not done at the same time between all subjects. Since the public health system in Medan has not run well, many patients experienced delayed of arrival at the tertiary hospital, also with different prehospital management. According to the researchers, this will affect the level of awareness of research subjects at admission. Second, GCS within the first hour after resuscitation will be affected by drug intoxication, sedative, neuromuscular blockade, and hemodynamic instability. Papa et al. (2010) also found the similar obstacles with this study. They found no association between UCHL-1 and GCS levels after resuscitation, but they found a relationship between UCHL-1 levels and the very first GCS in the first 24 hours. GCS in the early 24 hours may be a more consistent way of assessing the level of awareness, with the fact that the accuracy of determining GCS will increase if it is repeated.

There was no difference in UCHL-1 between moderate and severe TBI. From a study of literature conducted by researchers, there was no research to compare UCHL-1 levels between patients with mild and severe TBI. Nevertheless, in this study, the level of consciousness was assessed immediately after resuscitation based on GCS. GCS after self-resuscitation, as discussed above, has limitations in determining the level of consciousness. In this study, there was no significant association between UCHL-1 and mortality in the first three days as well as with duration of stay. An interesting study was conducted by Majetschak et al. (2005). They measured ubiquitin levels in CSF for seven days in six patients with head injury. They found that ubiquitin levels decreased progressively in patients who survived and increased in patients who died until death. Although in Majetschak study, the marker of ubiquitin used is not UCHL-1, but another subgroup of ubiquitin, it illustrates that the increase in protein family content associated with reduced outcomes.

Mondello et al. (2012) reported that UCHL-1>5.22 ng/mL is a strong predictor of death of severe TBI patients (OR 4.8). In this research, there is no logistic regression test, and the researchers did not determine the cut off mortality in the first three days. However, in this study, there is an extreme value that becomes an outlier (3.34 ng / mL). The subject of the study died on the third day. The main limitation of this study was the inability to prove that UCHL-1 is not byprocedure other cells. The existing literature study still leads us that this specific protein is in neurons. However, there should be a comparison of UCHL-1 levels between head injury and extracranial injury without head trauma. UCHL-1 is known to increase in patients with neurodegenerative diseases. The oldest research subjects in this study aged 54 years, is sufficiently prone to experience nerve degeneration disease. The weakness of this research is that the screening is by using anamnesis. The outcomes assessed in this study were only mortality within the first three days and duration of care. Both were short-term
outcomes. Researchers did not conduct long-term outcome analysis because of difficulties in follow-up.

5. Conclusion
UCHL-1 levels of moderate and severe TBI were significantly higher than those with mild TBI. No significant correlation was between UCHL-1 levels and duration of treatment. No meaningful relationship was between UCHL-1 levels and initial GCS after resuscitation. There were no significant differences in UCHL-1 levels among subjects who died within the first three days compared to the survivors.

References
[1] Hyder A A, Wunderlich C A, Puvanachandra P, Gururaj G and Kobusingye O C 2007 The impact of traumatic brain injuries: a global perspective Neurorehabilitation 22 341-53
[2] Thurman D J, Alverson C, Dunn K A, Guerrero J and Sniezek J E 1999 Traumatic brain injury in the United States: A public health perspective J. Head Trauma Rehabil. 14 602-15
[3] Bener A, Omar A O, Ahmad E, Al-Mulla F H and Rahman Y S 2010 The pattern of traumatic brain injuries: a country undergoing rapid development Brain Inj. 24 74-80
[4] Bishop P, Rocca D and Henley J M 2016 Ubiquitin c-terminal hydrolase L1 (UCH-L1): structure, distribution and roles in brain function and dysfunction Biochem. J. 473(16) 2453-62
[5] Liu Y 2002 The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility Cell 111 209-18
[6] Koboissy F H, Ottens A K and Zhang Z 2006 Novel differential neuroproteomic analysis of traumatic brain injury in rats Mol. Cell. Proteomics 5 1887-98
[7] Blyth B J, Farahvar A, He H, Nayak A, Yang C and Shaw G 2011 Elevated serum ubiquitin carboxy-terminal hydrolase L1 is associated with abnormal blood brain-barrier function after traumatic brain injury J. Neurotrauma 28 2453-62
[8] Zetterberg M, Sjolander A, von Otter M, Palmer M S, Landgren S, Minthon L, et al. 2010 Ubiquitin carboxy-terminal hydrolase L1 (UCHL1) S18Y polymorphism in Alzheimer's disease Mol. Neurodeg. 5 11-5
[9] Mondello S, Linnet A and Buki A 2012 Clinical utility of serum levels of ubiquitin c-terminal hydrolase as a biomarker for severe traumatic brain injury Neurosurgery 70 666-75
[10] Papa L, Akinyi L, Liu M C and Pineda J A 2010 Ubiquitin c-terminal hydrolase is a novel biomarker in humans for severe head injury Crit. Care Med. 38 138-44