Analysis of Neuron-Specific Enolase of Cerebrospinal Fluid and Post Mortem Serum of Blunt Head Trauma in Cause and Time of Death Determination

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Abstract. Blunt head trauma is the highest cause of death in criminal crime. Various attempts have been conducted to find alternative substitutes for the autopsy to determine the cause of death. NSE levels have been used as biomarkers in blunt head trauma in a living patient. The purpose of this study was to analyze NSE levels of CSF and serum in cause and time of death determination. This study was an experimental study with a post-test only group design, with the treatment of death due to blunt head trauma compared to deaths from acute ketamine poisoning. The number of treatment groups was 8, with samples of each group were 6 adult Sprague Dawley Rattus novergicus. NSE levels were examined at 0 hours, 1 hour, 2 hours and 3 hours after death. Analysis of NSE levels of CSF and serum in determining the cause and time of death was undertaken with the General Linear Model Repeated Measure test. We found increases in NSE levels of CSF and serum in both groups of deaths due to blunt head trauma and acute ketamine poisoning, and there was a significant difference between the two groups with $p = 0.005$ of serum NSE levels. There was no significant difference in the time of death between NSE levels of CSF and serum. Overall, there are increases in NSE levels of CSF and serum in deaths due to blunt head trauma and acute ketamine poisoning.

Keywords: Blunt Head Trauma, NSE, Cause of Death, Time of Death Determination.

1 Introduction

Blunt head trauma is an important issue in forensic medicine as it is the most common cause of death due to criminal crime and is also known as the most numerous case of death in the world [1]. In 2002, the trauma caused 4.5 million deaths or 1 in 10 deaths worldwide. Various cases could induce a blunt head, yet the highest number is due to traffic accident [1],[2],[3].

In the case of investigation for criminal acts, determining the cause of death is very urgent for law enforcement officers. Determination of the cause of death should be conducted by performing an internal examination (autopsy), but the examination received much rejection from the public [7].

Autopsy rejection rates in Indonesia, especially in West Sumatra are very high. At M. Djamil Padang Hospital, almost 80% of families resisted autopsy, and the most common reason was they were unwilling the body manipulation. Autopsy refusal is not only happened in Indonesia, worldwide, but the number of autopsies also decreased by 40-50%. According to Burton et al. and Corona et al. and Cit Stawicki et al., in the United States, the number of

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autopsies decreased by 41% of all bodies that should have been autopsied in 1960 to 5-23% [8],[9].

Conventional procedures to estimates of the time of death are often carried out by examining changes in the body of the victim, including bruising, corpse stiffness, temperature changes, decay, stomach contents of the body. According to Dimaio et al., all those parameters used are untrustworthy and inaccurate. Several studies related to the time of death estimation continue to be performed by more objective techniques such as biochemical, histology and serology examination of various body fluids and body tissues. The body fluids that are common to be studied are blood, vitreous, cerebrospinal fluid, pericardium fluid and synovial fluid [12],[17],[18],[19].

Head injuries will cause several abnormalities such as hypoxia/brain ischemia, brain edema, metabolic disorders, changes in vascular permeability, reduced blood flow, inflammation, extensive axonal injury and increased intracranial pressure [27],[28]. All the changes that occur will cause damage or even death from other cells and brain tissue, which cell damage will cause an increase in several enzymes. Increased enzymes in head trauma have been used as biomarkers in clinical settings.

Neuron-specific enolase (NSE) is the main glycolytic enzymes found in the cytoplasm of neurons and also can be found in small amounts on platelets and red blood cells. Specific enolase neurons are a marker of neuronal death, and they increase after head trauma at all severity degrees. Elevation of serum NSE levels is correlated to the increase in intracranial pressure [29]-[33].

In this study, NSE levels in cerebrospinal fluid and postmortem serum in mortality due to blunt head trauma and death due to acute ketamine poisoning were being correlated to the determination of cause and time of death.

2 Method

This research was an experimental study with a post-test only group design. The treatment to treatment group rats was conducting blunt trauma to the head and euthanasia by injecting lethal dose ketamine, then undergoing an enzyme examination after the rat died. Measurements of enzyme levels for both groups were carried out at 0 hours after death, 1 hour after death, 2 hours after death and 3 hours after death.

3 Result

Cerebrospinal fluid NSE levels in deaths due to blunt head trauma and death from acute ketamine poisoning were described in Table 1.

Table 1. CSF NSE levels in deaths from blunt head trauma and acute ketamine poisoning based on the time of death.

Time of death	Group	n	Mean ± SD (ng / ml)
0 hours	Acute ketamine poisoning	6	29.9 ± 8.4
	Blunt head trauma	6	37.6 ± 7.6
1 hour	Acute ketamine poisoning	6	18.1 ± 6.9

	Blunt head trauma	6	24.6 ± 11.0
2 hours	Acute ketamine poisoning	6	36.2 ± 14.5
	Blunt head trauma	6	51.5 ± 20.6
3 hours	Acute ketamine poisoning	6	37.3 ± 24.3
	Blunt head trauma	6	22.2 ± 7.0

In Table 1, the mean of CSF NSE levels was higher in deaths due to blunt head trauma than deaths due to acute ketamine poisoning at 0 hours, 1 hour and 2 hours. Whereas at 3 hours after death, CSF NSE levels were higher in deaths due to acute ketamine poisoning than deaths due to blunt head trauma.

To see a graph of changes in CSF NSE levels based on the time of death and method of death can be seen in Figure 1.

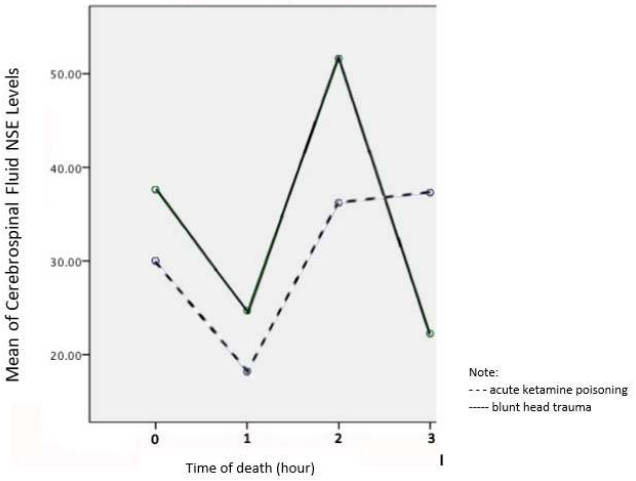

Fig. 1. Changes in LCS NSE levels based on time and method of death.

To see whether the data is normally distributed, a normality test was conducted following *Shapiro Wilk*, p> 0.05 in 6 data groups and p <0.05 in the death data group after an hour blunt head trauma and death after 3 hours acute ketamine poisoning. It is concluded that data is not normally distributed. Therefore, the General Linear Model (GLM) test requirements for repeated observations of the two groups are not fulfilled.

To see the difference in CSF NSE levels between deaths due to acute ketamine poisoning and the death due to blunt head trauma at 0 hours and 2 hours (normal data distribution), an unpaired t-test was performed.

Table 2. Differences in LCS NSE Periods of group manner of death.

Time of death	Manner of death	n	Mean ± SD (ng/ml)	P
0 hours	Acute ketamine poisoning	6	29.9 ± 8.4	0.132
	Blunt head trauma	6	37.6 ± 7.6	
2 hours	Acute ketamine poisoning	6	36.2 ± 14.5	0.166
	Blunt head trauma	6	51.5 ± 20.6	

In Table 2, the unpaired t-test for 0 hours obtained p = 0.132 and 2 hours obtained p = 0.166. It was concluded that there was no significant difference in CSF NSE levels in deaths from acute ketamine poisoning and death due to blunt head trauma at 0 hours and 2 hours of the time of death.

Serum NSE levels in deaths due to blunt head trauma and death due to acute ketamine poisoning can be observed in Table 3.

Table 3. Serum NSE levels in deaths due to blunt head trauma and acute ketamine poisoning based on time of death.

Time of death	Kelompok	n	Mean ± SD (ng/ml)
0 hours	Acute ketamine poisoning	6	4.6 ± 0.6
	Head blunt trauma	6	4.7 ± 0.3
1 hour	Acute ketamine poisoning	6	5.3 ± 0.4
	Head blunt trauma	6	5.1 ± 0.7
2 hours	Acute ketamine poisoning	6	6.7 ± 0.2
	Head blunt trauma	6	5.7 ± 1.4
3 hours	Acute ketamine poisoning	6	5.5 ± 0.7
	Head blunt trauma	6	4.0 ± 0.2

In Table 3, the mean of serum NSE levels of acute ketamine poisoning was higher in deaths from than deaths due to blunt head trauma, except at 0 hours of the time of death.

In Figure 2, a graph shows changes in serum NSE levels based on time and method of death.

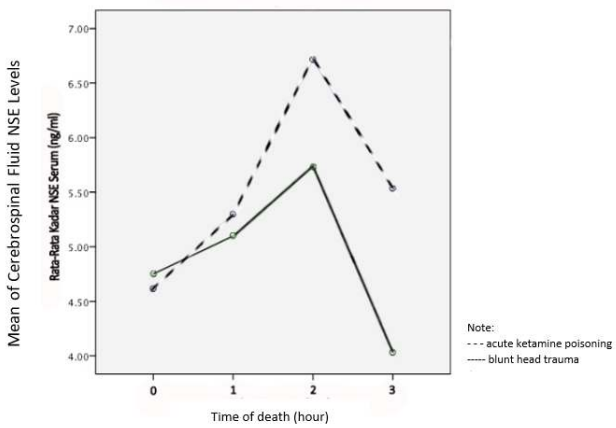

Fig. 2. Changes in serum NSE levels based on time and manner of death

Furthermore, a normality test was performed by Shapiro Wilk, obtained p-value > 0.05 in all groups of data, so it was concluded that data were normally distributed.

To see differences in serum NSE levels based on time and manner of death, the General Linear Model (GLM) test was performed for repeated observations of the two groups.

Table 4. Multivariate test results for changes in serum NSE levels based on time of death and method of death

	F	P
Time of death (0,1,2,3 hours)	6.438	0.016
Manners of death (acute ketamine poisoning, blunt head trauma)	4.061	0.037

In the multivariate test results, there were significant differences in serum NSE levels based on the time of death with a value of $p = 0.016$. There were significant differences in serum NSE levels in deaths due to acute ketamine poisoning with death due to blunt head trauma ($p = 0.037$).

The difference in serum NSE levels based on the time of death both in the death group due to blunt head trauma and death due to acute ketamine poisoning can be observed in the result of tests of within-subject contrasts in Table 5.

Table 5. Changes in serum NSE levels based on time of death.

Comparison of time of death	F	p
1 hour vs 0 hours	3.079	0.110
2 hours vs. 0 hours and 1 hour	17.422	0.002
3 hours vs. 0 hours, 1 hour and 3 hours	8.208	0.017

In Table 5, the test results in accordance with the measurement of time of death for deaths due to acute ketamine poisoning and death due to blunt head trauma showed no significant difference in serum NSE levels between 1 hour mortality and 0 hours ($p > 0.05$), and there were significant differences in serum NSE levels between 2 hours of death time and 0 hours and 1 hour; and between the time of death 3 hours and 0 hours, 1 hour and with 2 hours ($p < 0.05$).

Table 6. Changes in serum NSE levels affected by manners of death

Manner of death	F	p
Acute ketamine poisoning Blunt head trauma	13.179	0.005

Table 6 is the result of the test of between-subject effects, showed $p = 0.005$ ($p < 0.05$) which meant there were significant differences in serum NSE levels between deaths due to acute ketamine poisoning and death due to blunt head trauma, where serum NSE levels were higher in deaths due to acute ketamine poisoning.

4 Discussion

The rate of CSF NSE in the group of death due to blunt head trauma was higher than in the group of deaths due to acute ketamine poisoning, except in 3 hours after death. There was not any significant difference in CSF NSE levels in deaths from acute ketamine poisoning and death due to blunt head trauma. In both groups, CSF NSE levels increased from normal.

Research by Hans et al. obtained LCS NSE levels in cisterna magna samples in normal living mice with <4.4 ng/ml. According to Palmio, CSF NSE levels in the normal human population are 17.34,6 ng/l. Casimiro et al. in his study of the normal population, found CSF NSE levels were twice as high as serum NSE levels. Ondruschka et al. concluded that the higher the NSE level, the smaller the time of death with trauma. According to Siman, NSE levels are used as a marker of acute brain damage. An increase in NSE levels corresponds to the severity of brain damage [31,35,41-47].

The average change in LCS NSE levels based on the time of death cannot be used to estimate the time of death.

In this study, the mean postmortem serum NSE levels were higher in deaths due to acute ketamine poisoning than deaths due to blunt head trauma, except at 0 hours of death. There were significant differences in serum NSE levels in both groups. NSE levels of deaths due to acute ketamine poisoning and death due to blunt head trauma increased from normal.

Chekhonim et al. found normal serum NSE levels in mice ranged from 1.7 ± 1.3 ng/ml. Research by Skogseid et al., Zahra et al. and Olivecrona et al. concluded, in patients who had a head injury an increase in serum NSE levels were related to the severity of brain injury. According to Skogseid et al., NSE is a marker of acute brain damage [34,48].

In the table of within-subject contrasts test, it was concluded that changes in serum NSE levels based on the time of death could not be used as a basis for estimating the time of death because changes in serum NSE levels were not significant at all levels of time of death with the time of previous death.

Enolase is the main enzyme for energy metabolism found in all cell cytoplasm [49]. NSE involved in the glycolysis pathway in the change of 2-phosphoglycerate to phosphoenolpyruvate at the ninth stage of the glycolysis process. In head injury, there is an increase in intracell calcium levels which will cause intracellular catabolic processes [31,42,50-52].

According to Brunswick et al. and Zetterberg et al., increased activity of various membrane pumps to restore ion balance causes increased glucose consumption, reduction in energy storage, Ca2 + enter mitochondria, imbalance of oxidative metabolism and glycolysis with lactate production, so that in this process, NSE cannot function [36,53]. In the case of neuron damage, the excessive enzyme is not functional because the aerobic glycolysis process decreases.

In this study, it was found that NSE levels were higher than normal levels with 6 times higher than serum levels of NSE. In a study of normal population, CSF NSE levels were twice higher than serum NSE levels [54]. CSF NSE levels increased 6 times higher than serum possibly due to blood contamination in CSF fluid when sampling, high levels of LCS NSE indicated that NSE was released in brain trauma. NSE is a marker of increased neuronal trauma after focal or diffuse ischemia [49]. In this research, NSE levels increases were mostly due to neuronal cell damage by direct trauma. Besides, the increases also influenced by the process of cell damage that began to occur in secondary injuries. In cell damage setting, NSE neurons are released out of the cytoplasm [50]. Neuronal damage and impaired blood-brain barrier integrity can be detected by NSE released into CSF and then into the blood. So it can be concluded that NSE levels can be used as biomarkers in deaths due to blunt head trauma [49,50].

5 Conclusion

There was an increase in postmortem cerebrospinal fluid NSE level in deaths due to blunt head trauma and acute ketamine poisoning, but there was no significant difference in correlation to the time of death. There was an increase in serum postmortem NSE level in deaths due to blunt head trauma and acute ketamine poisoning, but there was no significant difference in correlation to the time of death.

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