

## The Clinical Significance of Measuring the Level and Trend of Butyrylcholinesterase for Patients with *Gloydius blomhoffii* Bite

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### Original Research Article

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**Abstract:** We retrospectively conducted a medical chart review of all patients with mamushi bites who were treated in our department between July 2013 and August 2017. The subjects were divided into two groups: the Mild group included subjects whose grades of mamushi bites were I and II, while the Severe group included subjects whose grades of mamushi bites were III, IV, and V. The subjects' age, sex, medical history, duration from bite to arrival at the hospital, grade on arrival and the maximum grade of mamushi bites classification during hospitalization, hospital day of occurrence of maximum grade of mamushi bites, duration of hospitalization, ratio of reception of anti-venom, mortality rate, and the level of BCHE on arrival, at its minimum, and its trend within seven days were investigated and compared between the two groups. Thirty-five cases were examined in this study. The swelling associated with mamushi bites tended to be significantly worse after admission, even if the patients received treatment. The swelling associated with mamushi bites ceased within seven days of arrival. The BCHE level showed a significant decreasing trend after admission. The grade of mamushi bites on arrival, ratio of reception of anti-venom, diminution (level on arrival minus the minimum level of BCHE during hospitalization) of BCHE, and duration of hospitalization in the Mild group were significantly lower than in the Severe group. However, the minimum BCHE level in the Mild group was significantly higher than that in the Severe group. In addition, the minimum BCHE level had a moderate negative relationship with the maximum grade classification, while the diminution of BCHE had a moderate positive relationship with the maximum grade classification. Accordingly, BCHE may be useful for objectively and quantitatively evaluating patients with mamushi bites.

**Keywords:** *Gloydius blomhoffii*; butyrylcholinesterase; severity.

### INTRODUCTION

Humans have two cholinesterases: acetylcholinesterase (ACHE) and butyrylcholinesterase (BCHE) [1-3]. ACHE participates in cholinergic neurotransmission by hydrolyzing acetylcholine and is expressed in nerve and blood cells. BCHE primarily exists in blood plasma, where it serves as a bio-scavenger that protects ACHE in the nerve synapses from inhibition by organophosphorus toxicants. In addition to its use for indicating the severity of organophosphate poisoning, BCHE in blood has been used as a biomarker for the severity of chronic liver disease; severity of Parkinson's disease; ability to tolerate stress, inflammation, and oxidative stress in cases of chronic obstructive pulmonary disease; and the trauma-induced acute systemic inflammatory response [4-10].

The Japanese mamushi, *Gloydius blomhoffii*, is a species of pit viper found throughout Japan, except on the southeast islands [11]. One problem with treating

mamushi-bite patients is that few quantitative indicators reflecting the severity of the bite have been reported. Our institute measured the level of BCHE for patients including mamushi-bite to evaluate the liver function and nutritional status [12]. Accordingly, we retrospectively investigated the clinical significance of measuring the level and trend of BCHE for patients with mamushi bites.

### METHODS

The protocol of this retrospective study was approved by our institutional review board, and the examinations were conducted according to the standards of good clinical practice and the Declaration of Helsinki.

We retrospectively conducted a medical chart review of all patients with mamushi bites who were treated in our department between July 2013 and August 2017. The exclusion criteria were patients whose level of BCHE had not been measured and who

were not admitted to our hospital. Mamushi bites were diagnosed by witnessing a mamushi and/or based on the appearance of wounds resulting from biting and clinical symptoms, as the main island of Japan has only two poisonous snakes (mamushi and yamakagashi), and the associated bite wounds and clinical symptoms differ completely between them[13,14].

Grade classification for mamushi bites was used to determine the severity of injuries, as follows: Grade I, redness and swelling around the bitten area; Grade II, redness and swelling, including the wrist or foot joint; Grade III, redness and swelling of the elbow or knee joint; Grade IV, redness and swelling of the whole extremity; and Grade V, redness and swelling beyond the extremities or exhibiting systemic symptoms. Grades of mamushi bites are rather complicated, and Hifumi *et al.* combined the 5 grades of mamushi bites into 2 groups: mild (grades of mamushi bites I and II) and severe (grades of mamushi bites III, IV, and V)[15,16].

Our protocol for treating patients with mamushi bites involves the intravenous administration of cepharanthine with toxoid for tetanus and antibiotics for mild cases and additional anti-venom for severe cases. The patients were admitted until improvement or the lack of further deterioration of the swelling and pain in the affected extremities could be confirmed. The vital signs, maximum swelling evaluated by the grade classification for mamushi bites, and findings of a biochemical analysis of the blood during hospitalization were examined every day for patients with mamushi bites.

The subjects were divided into two groups: the Mild group included subjects with grades of mamushi bites I and II, while the Severe group included subjects with grades of mamushi bites III, IV, and V. The subjects' age, sex, medical history, duration from bite to arrival at the hospital, grade on arrival and the maximum grade of mamushi bites classification during hospitalization, hospital day of occurrence of maximum grade of mamushi bites, duration of hospitalization, ratio of reception of anti-venom, mortality rate, and the level of BCHE on arrival, at its minimum, and its trend within seven days were investigated and compared between the two groups. In addition, we analyzed the correlation between the maximum grade classification and the BCHE level on arrival, at its minimum, and its diminution. The diminution was calculated using the

minimum level during hospitalization and the level on arrival.

The data were expressed as the mean  $\pm$  standard deviation (SD) or median (interquartile range) for continuous variables, and the number (percentages) for categorical variables. P values of  $<0.05$  were considered to indicate statistical significance.

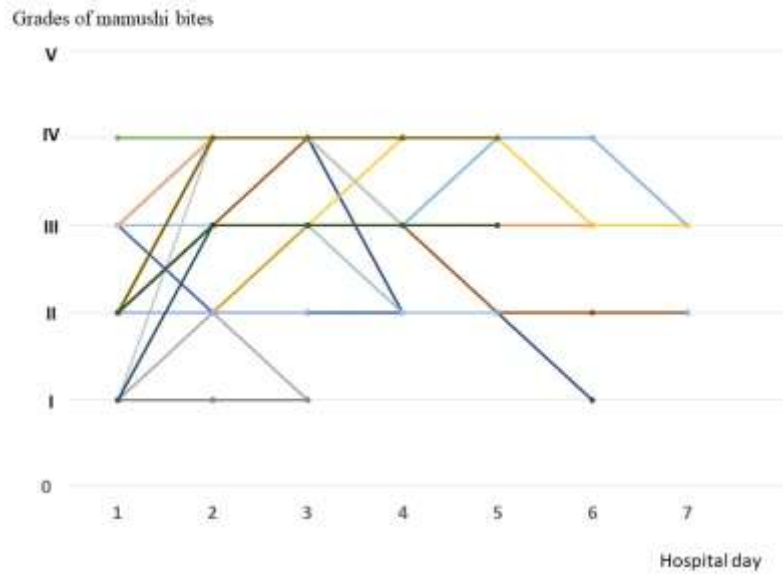
## **RESULTS**

There were 36 cases diagnosed with mamushi bites during the investigation period. Among them, one patient was transported to another hospital without an examination of blood test due to all beds being occupied. After excluding this case, 35 cases were treated as subjects.

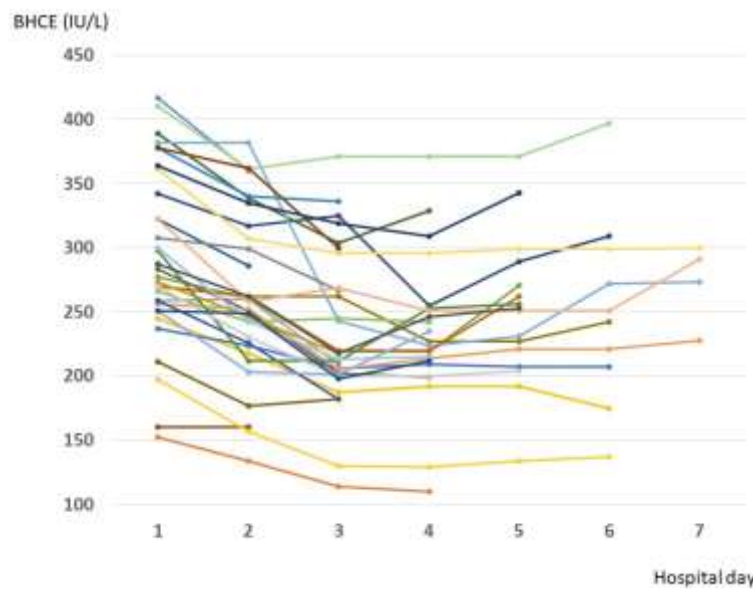
Table 1 shows the background of subjects. The subjects did not have any history or present illness of organophosphate poisoning, chronic liver disease, Parkinson's disease, chronic obstructive pulmonary disease, or trauma. The swelling associated with mamushi bites tended to be significantly worse after admission, even in those that received treatments, than before admission (Table 1 and Figure 1). The swelling associated with mamushi bites generally subsided within seven days of arrival. The BCHE level showed a significant decreasing trend after admission (Table 1 and Figure 2). There were no mortality cases among the subjects.

Table 2 shows the results of a comparison between the two groups. The female ratio in the Mild group was greater than that in the severe group, but not to a statistically significant degree. The BCHE level on arrival in the Mild group was greater than that in the severe group, but not to a statistically significant degree. The grade of mamushi bites on arrival, ratio of reception of anti-venom, diminution (level on arrival minus the minimum level of BCHE during hospitalization) of BCHE, and the duration of hospitalization in the Mild group were significantly lower than in the Severe group. However, the minimum BCHE level in the Mild group was significantly higher than that in the Severe group.

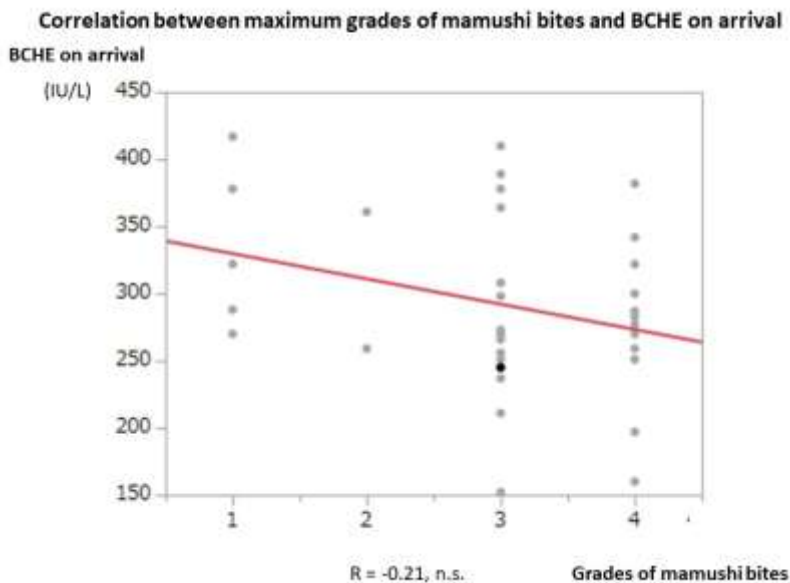
Figures 3-5 show the correlation between the maximum grade classification and BCHE level on arrival, at its minimum, and its diminution. The minimum BCHE level had a moderate negative relationship with the maximum grade classification, while the diminution of BCHE had a moderate positive relationship with the maximum grade classification.



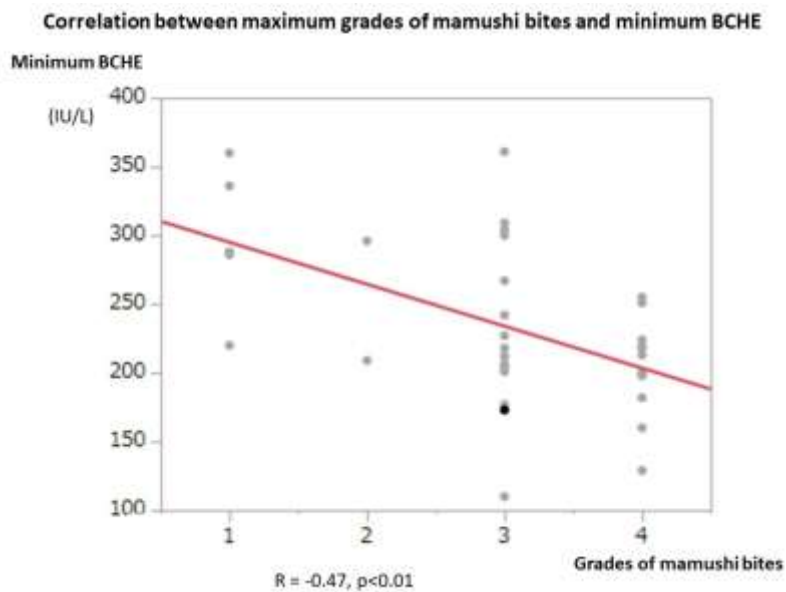
**Fig-1: Trend in the grade of mamushi bites over seven days. Swelling of mamushi bites tended to be significantly worse after admission than before, even in those who received treatments**



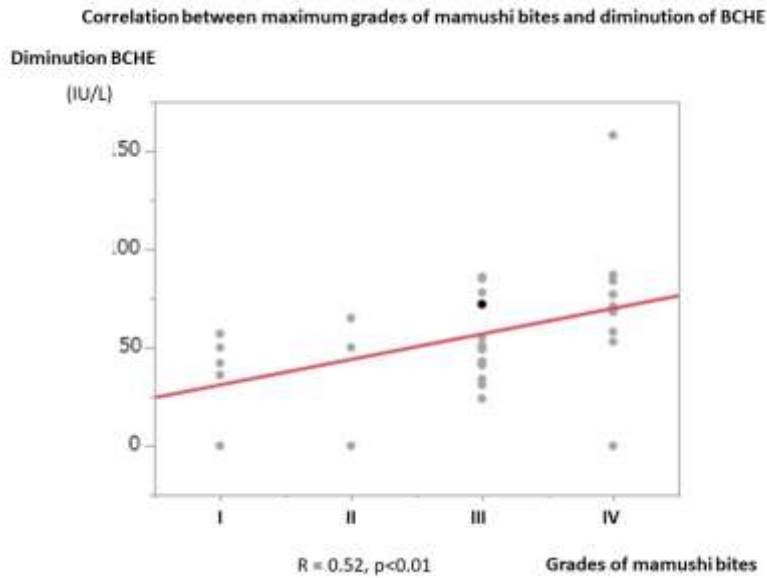
**Fig-2: Trend in the level of BCHE over seven days. The BCHE showed a significant decreasing trend after admission**



**Fig-3:** Correlation between the maximum grade classification and the BCHE level on arrival. A weak correlation was noted between the maximum grade classification and the BCHE level on arrival, although not a significant correlation. BCHE: butyrylcholinesterase



**Fig-4:** Correlation between the maximum grade classification and the minimum BCHE level. A significant moderate negative correlation was noted between the maximum grade classification and the minimum BCHE level. BCHE: butyrylcholinesterase



**Fig-5: Correlation between the maximum grade classification and the diminution of the BCHE level. A significant moderate positive correlation was noted between the maximum grade classification and the minimum BCHE level. BCHE: butyrylcholinesterase**

**Table-1: Subjects' background characteristics**

Number of subjects	35
Age (years)	12-82 (61.5 ± 18.5)
Sex (Male/Female)	18/17
Duration from bite to arrival	
The same day	33
The same day	2
Grade I on arrival	9
Maximum	5
II on arrival	9
Maximum	5
II on arrival	18
Maximum	5
II on arrival	18
Maximum	3
III on arrival	7
Maximum	15
IV on arrival	1
Maximum	12
V on arrival	0
Maximum	0
On arrival (Median)	2 (1)
Maximum (Median)	3 (1)
On arrival vs. Maximum	p < 0.0001
Hospital day of occurrence of maximum grade of mamushi bites	2-7 (4.0 ± 1.3)
Anti-venom (%)	25 (71.4)
Hospitalization (day)	2- 13 (5.0 ± 2.5)
BCHE on arrival	2- 13 (5.0 ± 2.5)
Minimum	110-361 (233 ± 10)
On arrival vs. Minimum	p < 0.0001
Mortality (%)	0

**Table-2: Results of the comparison between the two groups**

	Mild	Severe	p-value
Sex (Male/Female)	6/2	12/15	0.1
Age (years)	61.7 ± 18.0	61.2 ± 19.0	n.s.
Grades of mamushi bites D1	I	II	<0.001
Anti-venom (yes/no)	1/7	24/3	<0.0001
Maximum grade of grades of mamushi bites	3.8 ± 1.7	4.1 ± 1.2	n.s.
BCHE D1(IU/L)	327 ± 59	282 ± 65	0.1
D1-Min (IU/L)	37 ± 24	62 ± 28	<0.05
Duration of hospitalization	3.5 ± 1.8	5.4 ± 2.6	<0.05

BCHE: butyrylcholinesterase, D1: on arrival, Min: minimum

## DISCUSSION

This is the first report showing that the BCHE level in patients with mamushi bites tended to decrease, and the minimum BCHE level and its diminution were associated with the maximum grade of mamushi bite.

The present study did not include patients with any history or present illness of organophosphate poisoning, chronic liver disease, Parkinson's disease, chronic obstructive pulmonary disease, or trauma. However, some of the subjects might have had wound infection, which can reduce the BCHE level. Such an infection might have therefore influenced the level of BCHE in the present study.

One cause of the decrease in the BCHE level might have been a direct effect of the venom. Fasciculins are a class of toxic proteins found in certain snake venoms, notably some species of mamba [17,18]. Fasciculins from mambas inhibit intensely mammalian and fish ACHE and weakly inhibit BCHE [17-19]. The mechanism is similar to the effect of organophosphate poisoning. While there is no evidence at present that mamushi venom contains fasciculins or similar substances, if their venom does indeed contain fasciculins, this may explain the decrease in the BCHE level of patients suffering from mamushi bites.

The present study failed to demonstrate any statistical significance associated with the BCHE level on arrival for patients with mamushi bites. However, the minimum BCHE level and the diminution of the BCHE level were found to be associated with the maximum grade of mamushi bite. Admittedly, observing trends in biomarkers to assess the severity of mamushi bites takes precious time. However, it may nevertheless be useful to evaluate the severity of mamushi bites objectively and quantitatively in addition to a simple visual observation of the patient.

The present study is associated with some limitations. First, the study investigated only mamushi bites and did not investigate poisonous snakes in other parts of the world, so the results of this study cannot be applied outside of Japanese mamushi. Second, this study did not include severe cases that required intensive care or fatal cases, so these results may not

apply to more severe cases. The retrospective nature of this study and the small patient population hamper the exploration of such issues. Therefore, future prospective studies involving a greater number of patients are needed to corroborate or expand on our findings.

## CONCLUSION

This is the first report showing that the BCHE level in patients with mamushi bites tended to decrease, and the minimum BCHE level and its diminution were associated with the maximum grade of mamushi bite. Accordingly, the BCHE level may be useful for objectively and quantitatively evaluating patients with mamushi bites.

## Conflict of Interest

The authors declare no conflicts of interest in association with the present study.

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## REFERENCES

1. Lockridge O. Review of human butyrylcholinesterase structure, function, genetic variants, history of use in the clinic, and potential therapeutic uses. *Pharmacol Ther.* 2015 Apr;148:34-46.
2. Pohanka M. Cholinesterases, a target of pharmacology and toxicology. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.* 2011 Sep;155(3):219-29.
3. Masson P, Lockridge O. Butyrylcholinesterase for protection from organophosphorus poisons: catalytic complexities and hysteretic behavior. *Arch Biochem Biophys.* 2010 Feb 15;494(2):107-20.
4. Eddleston M, Eyer P, Worek F, Sheriff MH, Buckley NA. Predicting outcome using butyrylcholinesterase activity in organophosphorus pesticide self-poisoning. *QJM.* 2008

- Jun;101(6):467-74.
5. Temel HE, Temel T, Cansu DU, Ozakyol A. Butyrylcholinesterase activity in chronic liver disease patients and correlation with Child-Pugh classification and MELD scoring system. *Clin Lab*. 2015;61(3-4):421-6.
  6. Dong MX, Xu XM, Hu L, Liu Y, Huang YJ, Wei YD. Serum butyrylcholinesterase activity: A biomarker for Parkinson's disease and related dementia. *Biomed Res Int*. 2017;2017:1524107.
  7. Brimijoin S, Tye S. Favorable impact on stress-related behaviors by modulating plasma butyrylcholinesterase. *Cell Mol Neurobiol*. 2017 Jul 15.
  8. Sicinska P, Bukowska B, Pajak A, Koceva-Chyla A, Pietras T, Nizinkowski P, Gorski P, Koter-Michalak M. Decreased activity of butyrylcholinesterase in blood plasma of patients with chronic obstructive pulmonary disease. *Arch Med Sci*. 2017 Apr 1;13(3):645-651.
  9. Zivkovic AR, Schmidt K, Sigl A, Decker SO, Brenner T, Hofer S. Reduced serum butyrylcholinesterase activity indicates severe systemic inflammation in critically ill patients. *Mediators Inflamm*. 2015;2015:274607.
  10. Zivkovic AR, Bender J, Brenner T, Hofer S, Schmidt K. Reduced butyrylcholinesterase activity is an early indicator of trauma-induced acute systemic inflammatory response. *J Inflamm Res*. 2016 Nov 18;9:221-230.
  11. Hifumi T, Yamamoto A, Morokuma K, Ogasawara T, Kiriu N, Hasegawa E, Inoue J, Kato H, Koido Y, Takahashi M. Surveillance of the clinical use of mamushi (*Gloydius blomhoffii*) antivenom in tertiary care centers in Japan. *Jpn J Infect Dis*. 2011 Sep 1;64(5):373-6.
  12. Imaki M, Ohguri M, Iuchi A, Yoshida Y, Tanada S. Evaluation of the effects of various factors on the serum cholinesterase activity level. *Jpn J Health Hum Eco* 1995; 61(2) :104-14.
  13. Ishikawa K, Ohsaka H, Omori K, Obinata M, Mishima K, Oode Y, Yanagawa Y. Pregnant Woman Bitten by a Japanese Mamushi (*Gloydius blomhoffii*). *Intern Med*. 2015;54(19):2517-20.
  14. Hifumi T, Sakai A, Kondo Y, Yamamoto A, Morine N, Ato M, Shibayama K, Umezawa K, Kiriu N, Kato H, Koido Y, Inoue J, Kawakita K, Kuroda Y. Venomous snake bites: clinical diagnosis and treatment. *J Intensive Care*. 2015 Apr 1;3(1):16.
  15. Sakio H. Mamushi (viper) bite in Kensei General Hospital. *Rinsho Geka*. 1985;40:1295-7.
  16. Hifumi T, Yamamoto A, Morokuma K, Okada I, Kiriu N, Ogasawara T, Hasegawa E, Kato H, Inoue J, Koido Y, Takahashi M. Clinical efficacy of antivenom and cepharanthine for the treatment of Mamushi (*Gloydius blomhoffii*) bites in tertiary care centers in Japan. *Jpn J Infect Dis*. 2013;66(1):26-31.
  17. Harel M, Kleywegt GJ, Ravelli RB, Silman I, Sussman JL. Crystal structure of an acetylcholinesterase-fasciculin complex: interaction of a three-fingered toxin from snake venom with its target. *Structure*. 1995 Dec 15;3(12):1355-66.
  18. Jbilo O, Bartels CF, Chatonnet A, Toutant JP, Lockridge O. Tissue distribution of human acetylcholinesterase and butyrylcholinesterase messenger RNA. *Toxicon*. 1994 Nov;32(11):1445-57.
  19. Durán R, Cerveñansky C, Karlsson E. Effect of fasciculin on hydrolysis of neutral and choline esters by butyrylcholinesterase, cobra venom and chicken acetylcholinesterases. *Toxicon*. 1996 Aug 1;34(8):959-63.