Journal of Rural Medicine

Original article

Prognostic indicators among laboratory data on arrival to assess the severity of mamushi bites

Ikuto Takeuchi[1], Kazuhiko Omori[1], Hiroki Nagasawa[1], Kei Jitsuiki[1], Akihiko Kondo[1], Hiromichi Ohsaka[1], Kouhei Ishikawa[1], and Youichi Yanagawa[1]

[1]*Department of Acute Critical Care Medicine, Shizuoka Hospital, Juntendo University, Japan*

Abstract

Objective: This study aimed to retrospectively determine which laboratory data on arrival for patients with mamushi bites was useful to predict the severity of mamushi bites.

Materials and Methods: The subjects were divided into the following two groups: the mild group included subjects with mamushi bites Grades I and II, while the severe group included subjects with mamushi bites Grades III, IV, and V. The subjects' variables were compared between the two groups.

Results: There were no significant differences between the two groups regarding the levels of hematocrit, total protein, alanine aminotransferase, aspartate aminotransferase, creatinine phosphokinase, blood urea nitrogen, creatinine, and international normalized ratio of prothrombin time on arrival. Moreover, white blood cell count and platelet count on arrival in the mild group were significantly lower than those in the severe group. Furthermore, activated partial thromboplastin time on arrival was significantly higher in the mild group than in the severe group. Multivariate analysis using white blood cell count and platelet count and level of activated partial thromboplastin time revealed the following significant prognostic indicators of severity of mamushi bites: white blood cell count (Log Worth, 2.1; $p<0.01$) and platelet count (Log Worth, 1.6; $p<0.05$).

Conclusion: White blood cell count and platelet count on arrival of patients with mamushi bites are considered significant prognostic indicators in determining the severity of mamushi bites.

Key words: *Gloydius blomhoffii*, laboratory data, prognosis

(J Rural Med 2019; 14(2): 222–225)

Introduction

The Japanese mamushi, *Gloydius blomhoffii*, is a species of pit viper found throughout Japan, except on the southeast islands[1]. A mamushi bite has the following characteristics: one or two small wounds induced by venom fangs or one or two linear wounds produced by the withdrawing motion of the teeth. Mamushi bites cause swelling and pain that gradually spread from the bite site. A total of 1,000 cases of mamushi bites, resulting in 10 deaths, are estimated to occur annually[1, 2]. Causes of mortality include circulatory collapse, respiratory insufficiency, bleeding tendencies, and renal failure induced by rhabdomyolysis.

Mamushi venom consists of a variety of multiple enzymes that work as hemolytic toxins, including phospholipase A2, neurotoxins, and an alpha-toxin/a beta-toxin, that increase vascular permeability. Moreover, the venom contains arginine ester dehydrogenase that causes rhabdomyolysis, endopeptidase/bleeding factor (HR1 and HR2) that causes platelet aggregation, and L-amino acid oxidase[3–8].

The absence of prognostic indicators on arrival that determine the severity of a mamushi bite is one of the problems in treating patients with mamushi bites in the future. Early infusion of antivenom is recommended for patients with severe cases of mamushi bites; however, swelling, up to maximum swelling, in the site of a mamushi bite takes several days to develop. Okamoto *et al.* previously reported that the maximum creatinine kinase (CK) level and white blood cell (WBC) count during the clinical course were prognostic indicators of severe cases of mamushi bites[9, 10]. However, determining the prognostic indicators to assess the severity

Received: March 25, 2019
Accepted: July 8, 2019
Correspondence: Youichi Yanagawa, Department of Acute Critical Care Medicine, Shizuoka Hospital, Juntendo University, 1129 Nagaoka, Izunokuni-shi, Shizuoka 410-2295, Japan
E-mail: yyanaga@juntendo.ac.jp

of a mamushi bite is significantly time consuming. Accordingly, we retrospectively investigated the prognostic indicators of severity using the laboratory data on arrival for patients with mamushi bites.

Methods

The protocol of this retrospective study was approved by our institutional review board, and the examinations were performed according to the standards of Good Clinical Practice and the Declaration of Helsinki.

We retrospectively reviewed the medical charts of all patients with mamushi bites who were treated in our department between July 2013 and August 2017. The exclusion criteria were as follows: patients who had no laboratory data and patients who were not admitted in our hospital. Presence of mamushi bites were assessed and confirmed as follows: presence of wound caused by mamushi and patients presenting the clinical symptoms of a mamushi bite. The main island of Japan has only two poisonous snakes (mamushi and yamakagashi), and these snakes are totally different with respect to their bite characteristics, such as wounds, and clinical symtopms.

The following grade classification was used to determine the severity of injuries of mamushi bites: Grade I, redness and swelling around the bitten site; Grade II, redness and swelling in the wrist or foot joint; Grade III, redness and swelling in the elbow or knee joint; Grade IV, redness and swelling in the whole extremity; and Grade V, redness and swelling in parts beyond the extremity or presence of systemic symptoms. Since grade classification of mamushi bites is quite complex, Hifumi *et al.* combined Grades I–V into the following two groups: the mild (mamushi bites Grades I and II) and severe (mamushi bites Grades III, IV, and V) groups.

Patients with mamushi bites received the following treatment: intravenous administration of cepharanthine, toxoid for tetanus, and antibiotics for mild cases and antibiotics with antivenom for severe cases. The patients were admitted, with consistent follow-up and monitoring of their conditions, i.e., if their condition had either improved or not improved, on the basis of the assessment of swelling and pain in the affected extremities. The vital signs, the maximum swelling evaluated using the grade classification for mamushi bites, and the biochemical analysis of blood during hospitalization were basically examined every day for patients with mamushi bites.

The subjects were divided into the following two groups: the mild group included subjects with mamushi bites Grades I and II, while the severe group included subjects with mamushi bites Grades III, IV, and V. The subjects' age, sex, duration from a mamushi bite to the arrival to the hospital, grade on arrival and the maximum grade of mamushi bite during hospitalization, duration of hospitalization, ratio of reception of antivenom, mortality rate, and laboratory data on arrival (WBC count, hematocrit, platelet count, total protein, alanine aminotransferase, aspartate aminotransferase, creatinine phosphokinase, blood urea nitrogen, creatinine, activated partial thromboplastin time, and international normalized ratio of prothrombin time) within 7 days were investigated and compared between the two groups.

The data were expressed as the mean ± standard deviation or median (interquartile range) for continuous variables and the number (percentages) for categorical variables. Variables with significance levels of $p<0.05$ were included in the multivariate analysis. P values <0.05 were considered statistically significant.

Results

A total of 36 patients with mamushi bites were included in the study. Among them, one patient who did not undergo an examination of blood test was transported to another hospital because all the beds in the previous hospital were already occupied. After excluding this one patient, 35 patients were considered as the subjects.

Table 1 shows the background of the subjects. Swelling caused by mamushi bites significantly worsened after admission, regardless of the subjects receiving treatments, and the swelling subsided within 7 days. Mortality case in all the subjects was not noted.

Table 2 shows the results of comparison between the two groups. There were no significant differences between the two groups in terms of the levels of hematocrit, total protein, alanine aminotransferase, aspartate aminotransferase, creatinine phosphokinase, blood urea nitrogen, creatinine, and international normalized ratio of prothrombin time on arrival. Moreover, WBC count and platelet count on arrival were significantly lower in the mild group than that in the severe group. Furthermore, activated partial thromboplastin time on arrival in the mild group was significantly higher than that in the severe group.

Multivariate analysis using white blood cell and platelet counts and the level of activated partial thromboplastin time revealed the following significant prognostic indicators of severity of mamushi bites: WBC count (Log Worth, 2.1; $p<0.01$) and platelet count (Log Worth, 1.6; $p<0.05$). The receiver operating characteristic curve analysis was performed to determine the cutoff values for WBC and platelet counts, and it revealed that the cutoff values for WBC and platelet counts were 7,400/μl (area under the curve=0.82) and 197,000/μl (area under the curve=0.75), respectively.

Discussion

This is the first report demonstrating that WBC count

Table 1 Background of the subjects

Number	35
Age (mean ± SD)	12–82 (61.5 ± 18.5) years
Sex (male/female)	18/17
Duration from a mamushi bite to the arrival to the hospital	
The same day	33
The next day	2
Grade	
On arrival (median)	2 (interquartile range, 1–2)
Maximum (median)	3 (interquartile range, 3–4)
Antivenom (%)	25 (71.4)
Hospitalization (mean ± SD)	2–13 days (5.0 ± 2.5)
Mortality (%)	0

*Grade: On arrival vs. maximum. p<0.0001.

Table 2 Results of the analysis between the two groups according to the severity of injuries of mamushi bites

	Mild		Severe		p-value
	Mean	SD	Mean	SD	
White blood cell (/μl)	6,037	1,273	8,892	3,314	< 0.01
Hematocrit (%)	41	2	41	4	n.s.
Platelet (/μl)	17	4	21	4	0.03
Total protein (g/dl)	7	0.4	6.9	0.5	n.s.
Alanine aminotransferase (IU/L)	19	6	17	6	n.s.
Aspartate aminotransferase (IU/L)	22	2	23	5	n.s.
Creatinine phosphokinase (IU/L)	141	82	167	123	n.s.
Blood urea nitrogen (mg/dl)	16	6	16	5	n.s.
Creatinine (mg/dl)	0.84	0.4	0.75	0.32	n.s.
APTT (sec)	28.1	2.1	26.1	2.8	<0.01
PT-INR	0.97	0.04	1	0.05	n.s.

Mild group: mamushi bites of Grades I and II. Severe group: mamushi bites of Grades III, IV, and V. SD: standard deviation; APTT: activated partial thromboplastin time; PT-INR: international normalized ratio of prothrombin time.

and platelet count on arrival of patients with mamushi bites are considered prognostic indicators in determining the severity of mamushi bites.

The increase in WBC count after a mamushi bite is comparable to the increase in WBC count in patients with myocardial infarction, with WBC count as the blood parameter that should immediately be normalized[11]. These findings suggest that circulating leukocytes should be recruited into the circulation in large measure in response to local stress after a mamushi bite. A study stating that the venom of a snake directly increases WBC concentration has not been reported, and this hypothesis might be applied to our results.

Regarding platelet concentration, besides being key elements in hemostasis and thrombosis, platelets have an important role in inflammatory and innate immune response[12]. This activity is associated with the platelets' ability to recognize pathogens through the expression of Toll-like receptors and the secretion of various cytokines, chemokines, and growth factors stored within their granule[12]. The presence of these substances positively correlates with tissue edema[13]. Accordingly, high platelet concentration in addition to neutrophil count may exacerbate the edema induced by mamushi bites.

According to our data, CK levels had no significant effect on outcomes in our study. Okamoto *et al.* have reported that CK levels in a mamushi bite are associated with the severity of rhabdomyolysis. Glycoprotein HR1 greatly induced rhabdomyolysis[14]. Thus, lag time is defined as the time that is required for the injected HR1 to attach itself to the cell membrane and cause muscle damage. An earlier increase in CK levels may suggest direct injection of the venom into circulation. The difference between Okamoto's study and our present study was the ratio of antivenom injection. Okamoto *et al.* have reported that eight out of the 20 patients with severe cases (40%) of mamushi bites did not receive antiserum, while 3 out of the 27 patients with severe cases (11%) of

mamushi bites did not receive antiserum in our study.

The present study has some limitations. Firstly, the study only investigated mamushi bites and did not investigate the bites of all poisonous snakes in the world; hence, the results of this study cannot be applied to all poisonous snakes. Secondly, this study did not include patients with severe cases of mamushi bites who required intensive care or who had mortality case; thus, the results of this study cannot be applied to severe cases of mamushi bites. The retrospective nature of this study and the small patient population inhibit the exploration of such issues. Therefore, future prospective studies involving a greater number of patients are needed to further examine these limitations.

Conclusion

WBC count and platelet count on arrival of patients with mamushi bites are considered prognostic indicators in determining the severity of mamushi bites.

Funding

This work received funding from the Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities, 2015–2019, with the following title: The constitution of total researching system for comprehensive disaster, medical management, and correspondence to a wide-scale disaster.

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

Reference

1. Hifumi T, Yamamoto A, Morokuma K, *et al*. Surveillance of the clinical use of mamushi (Gloydius blomhoffii) antivenom in tertiary care centers in Japan. Jpn J Infect Dis 2011; 64: 373–376. [Medline]
2. Hifumi T, Yamamoto A, Morokuma K, *et al*. 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: 26–31. [Medline] [CrossRef]
3. Igari R, Iseki K, Abe S, *et al*. [Binocular diplopia and ptosis due to snakebite (Agkistrodon blomhoffi "mamushi")--a case report]. Brain Nerve 2010; 62: 273–277 (in Japanese, Abstract in English). [Medline]
4. Fujita M, Yamashita S, Kawamura Y, *et al*. Viper (Agkistrodon halys blomhoffii) bite with remarkable thrombocytopenia. Nihon Kyukyu Igakukai Zasshi 2005; 16: 126–130 (Journal of Japanese Association for Acute Medicine). [CrossRef]
5. Sun MZ, Liu S, Yang F, *et al*. A novel phospholipase A2 from Agkistrodon blomhoffii ussurensis venom: purification, proteomic, functional and structural characterizations. Biochimie 2009; 91: 558–567. [Medline] [CrossRef]
6. Ogawa Y, Murayama N, Fujita Y, *et al*. Characterization and cDNA cloning of aminopeptidase A from the venom of Gloydius blomhoffi brevicaudus. Toxicon 2007; 49: 1172–1181. [Medline] [CrossRef]
7. Kim JY, Lee SB, Kwon KR, *et al*. Isolation and Characterization of a 32-kDa Fibrinolytic Enzyme (FE-32kDa) from Gloydius blomhoffii siniticus Venom: Fibrinolytic Enzyme from Gloydius blomhoffii siniticus Venom. J Pharmacopuncture 2014; 17: 44–50. [Medline] [CrossRef]
8. Shioi N, Deshimaru M, Terada S. Structural analysis and characterization of new small serum proteins from the serum of a venomous snake (Gloydius blomhoffii). Biosci Biotechnol Biochem 2014; 78: 410–419. [Medline] [CrossRef]
9. Okamoto O, Sato S, Sakai T, *et al*. Comparative analysis of mamushi (*Gloydius blomhoffii*) bite patients indicates that creatinine kinase levels/white blood cell count trends reflect severity. Acute Med Surg 2015; 3: 120–127. [Medline] [CrossRef]
10. Okamoto O, Oishi M, Hatano Y, *et al*. Severity factors of Mamushi (Agkistrodon blomhoffii) bite. J Dermatol 2009; 36: 277–283. [Medline] [CrossRef]
11. Elliot MA, Eugene B. ST-segment elevation, Myocardial infarction. In: Fauci AS, Braunwald E, Kasper D (eds). Harrison's Principles of Internal Medicine, 17th ed. McGraw-Hill, New York, 2008; 1532–1544.
12. Carestia A, Kaufman T, Schattner M. Platelets: New bricks in the building of neutrophil extracellular traps. Front Immunol 2016; 7: 271. [Medline] [CrossRef]
13. Noma H, Mimura T, Yasuda K, *et al*. Role of soluble vascular endothelial growth factor receptor signaling and other factors or cytokines in central retinal vein occlusion with macular edema. Invest Ophthalmol Vis Sci 2015; 56: 1122–1128. [Medline] [CrossRef]
14. Oshima G, Omori-Sato T, Iwanaga S, *et al*. Studies on snake venom hemorrhagic factor I (HR-I) in the venom of Agkistrodon halys blomhoffi. Its purification and biological properties. J Biochem 1972; 72: 1483–1494. [Medline] [CrossRef]