Original article

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

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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

Introduction

The Japanese mamushi, Gloydius blomhoffii, is a species of pit viper found throughout Japan, except on the southeast islands. 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. 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/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.

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. However, determining the prognostic indicators to assess the severity...
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 symptoms.

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...
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\(^1\). 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\(^2\). 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\(^3\). The presence of these substances positively correlates with tissue edema\(^4\). 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 \textit{et al.} have reported that CK levels in a mamushi bite are associated with the severity of rhabdomyolysis. Glycoprotein HR1 greatly induced rhabdomyolysis\(^5\). 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 \textit{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

| 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.
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.

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

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