The Clinical Significance of C- Reactive Protein in Patients with *Gloydius blomhoffii* Bite

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**Purpose:** We retrospectively investigated the clinical significance of measuring the level of C-reactive protein (CRP) level in patients with mamushi (*Gloydius blomhoffii*) bites to differentiate it from bacterial cellulitis.

**Methods:** 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 patients were divided into two groups: the CRP–negative group included patients whose CRP levels were within the normal limit (<0.3 mg/dl) during hospitalization, while the CRP–positive group included patients whose CRP levels were over the normal limit (≥0.3 mg/dl) during hospitalization.

**Results:** Thirty-five cases were examined in this study. Seventeen patients were classified into the CRP-negative group and 18 were classified into the CRP-positive group. Only 2 of the patients (5%) had a positive CRP level on arrival. There were no significant differences in sex, ratio of delayed arrival, anti-venom administration, antibiotic administration, grade of mamushi bite on arrival and maximum grade of mamushi bite, ratio of increase in CRP over the normal limit, duration of hospitalization, or mortality rate between the two groups. However, the CRP-negative group tended to be younger than the CRP-positive group.

**Conclusion:** This is the first report to show that 5% of mamushi bite cases had a positive CRP level on arrival, and that half had a positive CRP level during hospitalization. Future prospective studies involving a greater number of patients are needed in order to determine whether or not a lack of an increase in the CRP level on arrival is a useful biomarker for differentiating between mamushi bites and cellulitis.

**Key words:** *Gloydius blomhoffii*, C-reactive protein (CRP), severity

**Introduction**

The Japanese mamushi, *Gloydius blomhoffii*, is a species of pit viper that is found throughout Japan, with the exception of the southeast islands. Mamushi bites cause swelling and pain that gradually spreads from the bite site, resembling bacterial cellulitis.

C-reactive protein (CRP) is a plasma protein with strong phylogenetic conservation and high resistance to proteolysis, which is predominantly synthesized in the liver in response to proinflammatory cytokines, especially IL-6, IL-1β, and TNF-α. It is well known that CRP may rise due to various processes causing inflammation, such as infection, collagen disease, inflammatory diseases (e.g., Crohn’s disease, psoriatic arthritis, systemic vasculitis, and Reiter’s disease), as well as due to necrosis after myocardial infarction and acute pancreatitis, trauma, and certain cancers. CRP is also used for the diagnosis and evaluation of the severity of soft tissue infection, including cellulitis. Soft tissue swelling with pain induced by mamushi bite is often misdiagnosed as cellulitis in Japan. It is important to administer anti-venom as early as possible for patients with a severe condition due to a mamushi bite in order to...
minimize the risk of mortality and sequelae.

Accordingly, we retrospectively investigated whether the CRP level would be useful for differentiating patients with mamushi bites from those with bacterial cellulitis.

**Methods**

The protocol of this retrospective study was approved by our institutional review board (approval number: 298), 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. Patients whose CRP level was not been measured and who were not admitted to our hospital were excluded from the present study. Mamushi bites were diagnosed based on witnessing a mamushi and/or the appearance of wounds resulting from a bite and clinical symptoms, as the there are only two species of poisonous snake on the main island of Japan (mamushi and yamakagashi), and the associated bite wounds and clinical symptoms of patients bitten by these snakes are completely different.7)

The severity of injuries in patients with mamushi bites was graded 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. The grades of mamushi bites are rather complicated, and Hifumi et al. combined the five grades of mamushi bites into two categories: mild (grades I and II) and severe (grades III–V) 9).

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 the 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 of mamushi bite, and the blood biochemistry findings during hospitalization were examined daily in patients with mamushi bites.

The patients were divided into two groups: the CRP-negative group included patients whose CRP level was within the normal limit (<0.3 mg/dl) during hospitalization, while the CRP-positive group included patients whose level of CRP was over the normal limit (≥0.3 mg/dl) during hospitalization. The following variables were evaluated: age, sex, duration from bite to arrival at the hospital (delayed or not [delay was defined as arrival at >24 h from the bite]), ratio of anti-venom administration, ratio of antibiotic administration, grade of mamushi bite on arrival and maximum grade during hospitalization, ratio of CRP over the normal limit on arrival, maximum CRP level during hospitalization, body temperature on arrival, maximum body temperature during hospitalization, white blood cell (WBC) count on arrival, maximum WBC count during hospitalization, creatine phosphokinase (CK) level on arrival, maximum CK level during hospitalization, duration of hospitalization, and mortality rate.

The data were expressed as the mean ± 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 of mamushi bite during the investigation period. Among them, one patient was transported to another hospital without a blood test because all hospital beds were occupied. After excluding this case, 35 patients were included in the present study. Twenty–three patients were diagnosed based on witnessing a mamushi, 11 were diagnosed by the shape of the bite wounds, and was diagnosed based on the clinical symptoms alone. Table-1 shows the results of the statistical analysis of the two groups. There were 17 patients in the CRP-negative group and 18 in the CRP-positive group. Only 2 or the patients (5%) had a positive CRP level on arrival. Regarding the 2 patients with a positive CRP level on arrival, 1 was a 70–year–old man who visited the hospital within 30 minutes after a mamushi bite, and the other was an 80–year–old woman who had been transported by an ambulance due to septic shock from cellulitis 1 day after a mamushi bite. One of these CRP-positive
patients underwent blood and wound culture studies, and the results were negative.

There were no significant differences in sex, ratio of delayed arrival, anti-venom administration, antibiotic administration, grade of mamushi bite on arrival and maximum grade of mamushi bite, ratio of increase in CRP over the normal limit, body temperature on arrival, maximum body temperature during hospitalization, WBC count on arrival, maximum WBC count during hospitalization, CK level on arrival, maximum CK level during hospitalization, duration of hospitalization, or mortality rate between the two groups. However, the CRP-negative group tended to be younger than the CRP-positive group.

**Discussion**

This is the first report to show that 5% of mamushi bite cases had a positive CRP level on arrival and half had a positive CRP level during hospitalization. Mamushi venom contains various enzymes that function as hemolytic toxins (e.g., phospholipase A2) or neurotoxins (e.g. alpha-toxin/ beta-toxin), increase vascular permeability (e.g., arginine ester dehydrogenase), cause rhabdomyolysis (e.g., endopeptidase/bleeding factor [HR1 or HR2]), and cause platelet aggregation (e.g., L-amino acid oxidase)\(^{10-15}\). However, mamushi venom has never been reported to cause an increase in the CRP level. In the present study, two patients had positive CRP levels on arrival. However, only one was CRP-positive within 30 minutes after a mamushi bite. Generally, the CRP level does not increase immediately after contracting a bacterial infection\(^{16}\). Accordingly, this patient may have had pre-existing inflammation before the mamushi bite. The other CRP-positive patient had delayed visiting a medical facility and was already complicated with cellulitis. Accordingly, the lack of an increase in the CRP level immediately after a mamushi bite might be a useful biomarker for differentiating between a mamushi bite and cellulitis induced by a bacterial infection\(^{16}\). However, the patients with positive and negative CRP levels did not show any marked clinical differences during

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Results of the analysis between two groups</th>
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<tbody>
<tr>
<td></td>
<td>CRP-negative</td>
</tr>
<tr>
<td>Sex</td>
<td>n=17</td>
</tr>
<tr>
<td>Male/Female</td>
<td>8/9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.0 ± 20.4</td>
</tr>
<tr>
<td>Delayed arrival (yes/no)</td>
<td>1/16</td>
</tr>
<tr>
<td>Anti-venom (yes/no)</td>
<td>11/6</td>
</tr>
<tr>
<td>Antibiotics (yes/no)</td>
<td>17/0</td>
</tr>
<tr>
<td>Grade of mamushi bite</td>
<td></td>
</tr>
<tr>
<td>On arrival</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Maximum</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Increase in CRP</td>
<td></td>
</tr>
<tr>
<td>On arrival (yes/no)</td>
<td>0/17</td>
</tr>
<tr>
<td>Maximum CRP</td>
<td>0.3 ± 0</td>
</tr>
<tr>
<td>Body temperature (℃)</td>
<td></td>
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<tr>
<td>On arrival</td>
<td>36.7 ± 0.4</td>
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<tr>
<td>Maximum</td>
<td>37.0 ± 0.3</td>
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<tr>
<td>White blood cell count (/μl)</td>
<td></td>
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<tr>
<td>On arrival</td>
<td>8,057 ± 2,310</td>
</tr>
<tr>
<td>Maximum</td>
<td>9,385 ± 3,520</td>
</tr>
<tr>
<td>Creatine phosphokinase (IU/l)</td>
<td></td>
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<tr>
<td>On arrival</td>
<td>168.3 ± 120.4</td>
</tr>
<tr>
<td>Maximum</td>
<td>313.0 ± 473.0</td>
</tr>
<tr>
<td>Duration of hospitalization</td>
<td>4.3 ± 1.7</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>0</td>
</tr>
</tbody>
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CRP, C-reactive protein
hospitalization; thus, this level may not influence the clinical outcome.

One cause of the increase in CRP among patients may be the induction of bacterial infection due to the snake bite. No reports have demonstrated that snake venom can directly increase the CRP level. While we found no evidence of a bacterial infection in a culture study in our investigation, snake bites may be complicated by bacterial infection, as the snake’s teeth may be contaminated with bacteria \(^{17-19}\). Snake bites can induce bacterial cellulitis, which can in turn induce an increase in the CRP level \(^{20, 21}\). Accordingly, the absence of an increase in the CRP might be a useful biomarker for differentiating between mamushi bites and cellulitis induced by bacterial infection, even during the follow-up of patients with mamushi bites.

Regarding the association between the CRP level and age, the CRP level tends to increase in older populations \(^{22}\). In addition, older individuals tend to develop opportunistic infections \(^{23, 24}\). Thus, the aging population may have affected the results of this study.

Our protocol for treating patients with mamushi bites involves the intravenous administration of antibiotics. However, no study has explored whether or not antibiotics have a beneficial effect after mamushi bites. Generally, animal bites are associated with the injection of bacteria into broken skin and prophylactic antibiotics may be required to prevent subsequent infection, in addition to proper wound care, such as inspection, debridement, and/or irrigation \(^{25}\). Some authors have recommended the routine prophylactic use of antibiotics after snake envenomation. However, others feel that antibiotic usage should only be initiated after noting clinical evidence of an infection, such as local tissue necrosis or gangrene, abscess formation, or bullae. As a result, the use of antibiotics for snake bite wounds is based on either a hospital's own policies or the primary interventions of individual clinicians \(^{26}\). As a mamushi bite can induce local tissue swelling and severe pain, resembling bacterial cellulitis, we routinely administer antibiotics for the treatment of mamushi bite wounds. Based on the results of the present study, the CRP level may be useful for identifying patients who need antibiotics for the treatment of bacterial cellulitis.

The present study is associated with some limitations. First, the diagnosis of a mamushi bite was dependent on the patient’s testimony, the shape of the bite wound, and/or clinical symptoms \(^{27}\). No reliable diagnostic biomarkers or methods for the definite diagnosis of venomous snake bites are available in clinical practice, so some patients might have suffered wounds induced by other causes \(^{28}\). Second, the study only investigated mamushi bites and did not investigate poisonous snakes in other parts of the world, so the results of this study cannot be applied to bites from other species of poisonous snake. Third, we did not evaluate trends in any other biomarkers. Fourth, we only performed culture studies in one patient; thus, the trends in the CRP level both with and without bacterial infection were not determined. Fifth, we did not compare the outcomes between the patients with isolated bacterial cellulitis and those with mamushi bite. Sixth, we did not measure the procalcitonin or presepsin levels because these analyses would need to be performed outside our institution and the results would not have been obtained on the same day. However, as procalcitonin and presepsin show high sensitivity in the diagnosis of bacterial infection, procalcitonin may be a useful tool for differentiating between a mamushi bite and cellulitis \(^{29, 30}\). The retrospective nature of this study and the small patient population hamper the exploration of such issues. Thus, future prospective studies involving a greater number of patients are needed to corroborate or expand on our findings.

**Conclusion**

This is the first report to show that 5% of mamushi bite patients had a positive CRP level on arrival and that half had a positive CRP level during hospitalization. The present results suggest that the absence of an increase in the CRP level after a mamushi bite might be a useful biomarker for differentiating between a mamushi bite and cellulitis induced by a bacterial infection. Future prospective studies involving a greater number of patients are needed in order to determine whether or not the lack of an increase in the CRP level on arrival is a useful biomarker for differentiating between a mamushi bite and cellulitis.

**Conflict of interest**

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

Funding

This work received funding from the Ministry of Education, Culture, Sports, Science and Technology (MEXT)–Supported Program for the Strategic Research Foundation at Private Universities, 2015–2019. The title is “[The constitution of total research system for comprehensive disaster, medical management, corresponding to wide-scale disaster].

References