

Original Article

Statistical analysis of anti-mamushi venom serum injection time and clinical course

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Aim: Early injection of anti-mamushi venom serum (antiserum) is believed to be effective for the treatment of patients with mamushi bites. However, there is no firm information that indicates the time range constituting "early" injection. We tried to quantify the cut-off time of antiserum injection that brings favorable clinical courses by clarifying the relationship between the injection time and clinical outcome.

Methods: We retrospectively analyzed the relationships between the time after bite, injection time of the antiserum, swelling grades, and laboratory values.

Results: The injection time of the antiserum in severe cases was significantly delayed as compared with non-severe cases. The best cut-off time of the antiserum injection that could distinguish non-severe and severe cases was 14 h. In the group that received the antiserum within 14 h, the antiserum injection may have successfully arrested the grade progression in a substantial number of cases. In the other group receiving the antiserum beyond 14 h, the grades in many cases possibly may have peaked by the time of antiserum injection.

Conclusion: The cut-off time of early injection for favorable clinical course was determined to be 14 h. A statistical basis concerning the appropriate antiserum injection time was made to help prevent a severe clinical course due to delayed injection.

Key words: *Gloydius blomhoffii*, mamushi bite, prevention of disease progression, timing of antiserum injection

INTRODUCTION

MAMUSHI (*Gloydius blomhoffii*) is a common viper in Japan, and its venom action is strong. A fraction of patients bitten by mamushi show symptoms such as renal failure and disseminated intravascular coagulation (DIC), requiring intensive care,^{1,2} and some patients even die.^{3,4} Anti-mamushi venom serum (antiserum) is regarded as the sole reagent that can neutralize mamushi venom.⁵

One previous report discussed the timing of antiserum injection, and described that injection within 3 h from the

bite suppressed the frequency of general symptoms.⁶ However, through our daily treatment of mamushi-bitten patients, we have observed that antiserum injection even beyond 3 h can still yield a good clinical outcome. Thus, although early antiserum injection is believed to prevent a severe clinical course, there is no firm information that indicates the time range constituting "early" injection, making the appropriate timing of antiserum injection confusing.

In the present study, we quantified the cut-off time of the antiserum injection that brings a favorable clinical course by analyzing the injection time–clinical outcome relationship.

METHODS

Study design

THIS STUDY WAS a retrospective observational study undertaken in Oita City Medical Association's Almeida Memorial Hospital and Oita Prefectural Hospital (Oita,

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Japan). The subjects were patients with mamushi bite who attended either hospital. Oita City Medical Association's Almeida Memorial Hospital is a primary care hospital (406 beds) that receives four to six patients with mamushi bites per year, and Oita Prefectural Hospital (566 beds) is a core hospital in Oita Prefecture that receives two to three mamushi-bitten patients per year. This study was approved by the ethics committee review board of Oita City Medical Association's Almeida Memorial Hospital and Oita Prefectural Hospital, which was organized according to the ethical guidelines for medical and health research involving human subjects developed by the Ministry of Health, Labor, and Welfare of Japan (approval no. 161 and 2-10, respectively). The antiserum (freeze-dried mamushi antivenom "Kaket-suken") was produced by The Chemo-Sero-Therapeutic Research Institute (Kumamoto, Japan).

Patients

We enrolled patients who had consulted Oita City Medical Association's Almeida Memorial Hospital and Oita Prefectural Hospital due to mamushi bite between 2005 and 2019. The diagnosis of mamushi bite was based on the identification of a mamushi snake by the patients or physicians or based on the characteristic swelling after the bite. Among the mamushi-bitten patients, those who received antiserum and whose final grades were known were eligible and further subjected to analyses. Patients who did not satisfy those criteria were excluded from this study.

Intervention

The grading score⁵ was used to express the swelling severity of mamushi bite (Table 1). The final grades were determined as follows: a final grade of 1–4 was determined when the peak of swelling was determined to be grade 1–4; a final grade of 5 was determined as soon as the clinical symptoms reached grade 5. Regarding patients' treatments,⁵ typically, incision/aspiration was done, and anti-tetanus agents and antibiotics were injected. Antiserum was injected with an index of grade 3; however, the decision to inject or not inject antiserum was ultimately left to the responsible physicians. Basically, all patients were admitted, and the clinical course was observed. After the bite grades and laboratory values were found ameliorating, the hospital treatment was terminated. For those presenting with a severe clinical course, additional intensive care was given. Severe cases were determined as those presenting with a final grade of 5, whereas non-severe cases were those presenting with a final grade of 1–4.

Table 1. Grades of swelling following mamushi bite

Grade	Swelling
1	Local
2	Hand/foot
3	Forearm/lower leg
4	Whole extremities
5	Involving the trunk and/or systemic symptoms

Data collection

The following clinical information was obtained from medical records: (i) patients' age, sex, and bite site (as demographic information); (ii) time from bite to arrival at the final hospital, time from bite to the antiserum injection, time from bite to reaching maximal grade, initial grade at arrival, grade at injection, final grade, presence or absence of anaphylaxis following antiserum injection, and cellulitis during treatment (as clinical outcomes); (iii) the presence or absence of incision/aspiration of the bitten area, usage of cepharanthine, steroid, anti-tetanus agents, and antibiotics (as intervention); and (iv) the maximal creatine kinase (CK) levels (as laboratory outcomes). The "final hospital" means the hospital where the antiserum was injected. Grades at the time of events are determined as follows: initial grade, grade at arrival at the final hospital; injection grade, grade at the time of the antiserum injection; and final grade, maximal grade throughout the clinical courses.

Statistical analysis

The data in each graph are expressed as raw, individual plots or as box-plots comprising the median, interquartile, and 10–90 percentile ranges (Figs S1 and S2), or as the median, average value \pm standard deviation. Categorical variables are expressed as numbers (%). Non-categorical variables between two groups were compared using the Mann–Whitney *U*-test (*U*-test) and the categorical variables were compared using Pearson's χ^2 -test or Fisher's exact test. *P*-values <0.05 were regarded as statistically significant.

All statistical analyses were undertaken using the Excel statistical software program file ystat 2006 (Igaku Tosho Shuppan, Tokyo, Japan). The cut-off value for the antiserum injection time was determined by making a receiver operating characteristic (ROC) curve and selecting the point nearest to an ideal point (sensitivity, 1.0; 1 – specificity, 0.0).

RESULTS

Patient profiles

A TOTAL OF 57 patients were enrolled, and their demographic characteristics are shown in Table 2. One severe case showed a lethal outcome,³ and five severe cases presented with symptoms such as renal failure, respiratory failure, and DIC (not shown). No significant differences in the gender, age, or site of bite were seen between the non-severe and severe cases. Treatments other than antiserum did not differ significantly between the two groups. Antibiotics were given in all cases but one, with penicillin and

cephem being given in 82% of those cases (data not shown). Intravenous steroid was given in 30 cases. Two cases of anaphylaxis and cellulitis were observed (Table 2). Thirty-six (85.7%) non-severe cases received antiserum at grades 3 and 4, whereas nine (60%) severe cases received the antiserum at grade 5, showing a significant difference between the two groups (Table 2).

Comparison of antiserum injection timing

The distribution of the antiserum injection time in the non-severe and severe cases is compared in Figure 1A and Table 2. The median injection time in the non-severe cases

Table 2. Demographics of 57 patients bitten by mamushi

	Non-severe	Severe	
Total number	42	15	
Sex			
Male	23 (54.8)	7 (46.7)	$P = 0.810000$
Female	19 (45.2)	8 (53.3)	
Age (years)			
Median	69.5	78	$P = 0.160000$
Average \pm SD	65.9 \pm 19.6	73.9 \pm 12.6	
Site of bite		(1 missing datum)	
Finger/hand	27 (64.3)	8 (53.3)	$P = 0.800000$
Arm	4 (9.5)	3 (20)	
Toe/foot	11 (26.2)	2 (13.3)	
Leg	0 (0.0)	1 (6.7)	
Treatments other than antiserum			
Incision/aspiration	32 (76.2)	10 (66.7)	$P = 0.990000$
Antibiotics	42 (100)	14 (93.3)	
Anti-tetanus agents	38 (90.5)	12 (80)	
Cepharanthine	36 (85.7)	11 (73.3)	
Intravenous steroid	23 (54.8)	7 (46.7)	
Grade at antiserum injection	(1 missing datum)		
1	1 (2.4)	0 (0.0)	$P = 0.000044^*$
2	4 (9.5)	0 (0.0)	
3	23 (54.8)	2 (13.3)	
4	13 (30.9)	4 (26.7)	
5	0 (0.0)	9 (60.0)	
Time of antiserum injection from bite			
Median (h)	4.125	20	$P = 0.000270^*$
Average \pm SD (h)	7.7 \pm 7.8	20.3 \pm 12.1	
Complication			
Anaphylaxis	1 (2.3)	1 (6.7)	$P = 0.460000$
Cellulitis	1 (2.3)	1 (6.7)	

Data are shown as n (%) unless otherwise indicated. Categorical variables, such as the sex and bite site, grade at serum injection, and other treatments, in non-severe and severe cases were compared by the χ^2 -test or Fisher's exact test, and non-categorical variables, such as the median ages and injection time, in non-severe and severe cases were compared by the U -test. Results are shown beside the panel. Due to some missing data, the case numbers do not correspond to those in the figures. The numbers of missing data are indicated in parentheses. *Statistically significant. SD, standard deviation.

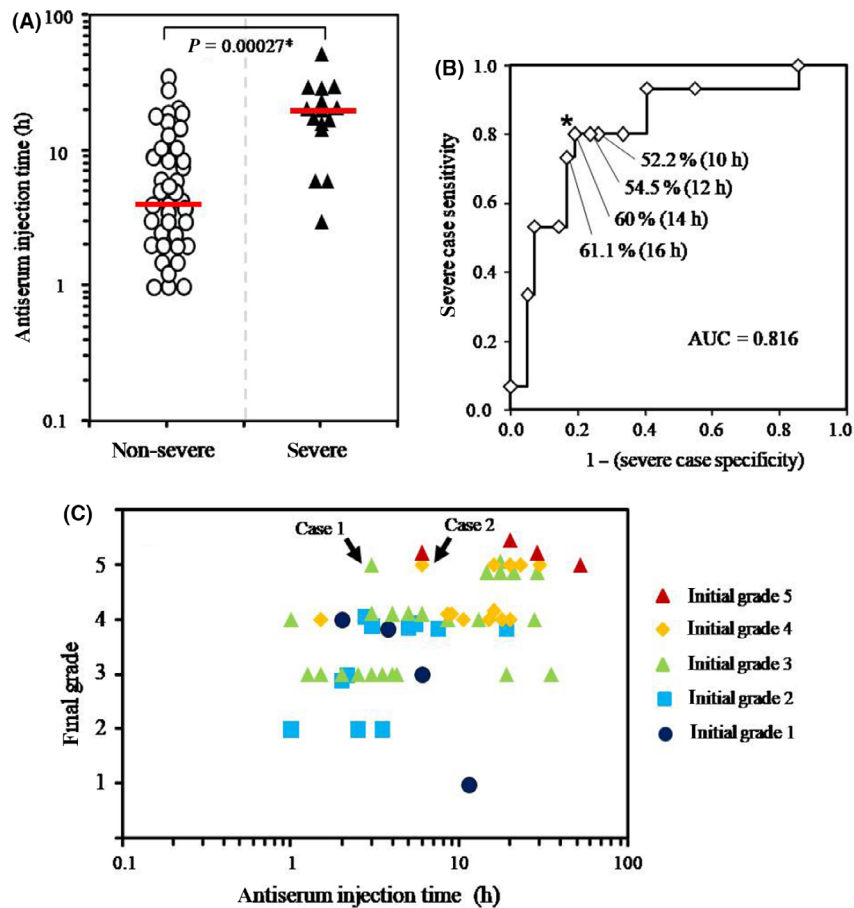


Fig. 1. Comparison of antiserum injection times between non-severe and severe cases of mamushi bite. A, Simple comparison of both groups. White circles and filled triangles indicate non-severe and severe cases, respectively. Median values are expressed by red bars. The P -value of the U -test is shown at the top of the panel. *Statistically significant. B, A receiver operating characteristic (ROC) curve. The sensitivity and specificity of severe cases at the cut-off time for every 2 h were calculated, and then a ROC curve was drawn. *Point nearest to an ideal point (sensitivity, 1.0; 1 – specificity, 0.0). The positive prediction values of severe cases are expressed as percentages, and each cut-off time is indicated in h. The value of the area under the ROC curve (AUC) is shown in the lower part of the panel. C, Relationship between antiserum injection time and final grades, and a comparison of initial and final grades. The position of each symbol represents the final grade indicated by a vertical bar. The five different symbols indicate initial grades of 1 to 5 (see figure key).

was 4.1 h after the bite, whereas that in the severe cases was 20 h (Table 2, $P = 0.00027$, U -test). Thus, the antiserum injection was significantly delayed in the severe cases. We tried to determine a good cut-off value for the antiserum injection time to distinguish non-severe and severe cases. A ROC curve for a severe case is shown in Figure 1B. With a cut-off time at 14 h (Fig. 1B, asterisk), the sensitivity and specificity of the severe cases were 80% and 81%, respectively, and a positive prediction value at this cut-off time was 60%. Based on the ROC curve, 14 h was deemed a favorable cut-off for the antiserum injection timing.

Next, the relationship between the antiserum injection timing and the final grades was evaluated (Fig. 1C). The

initial grade of each case is also indicated in Figure 1C. The final grade increased as the antiserum injection timing was delayed. Of note, approximately half of cases with initial grades of 1–4 appeared to show grade progression, regardless of the antiserum injection timing.

Grade progression before and after antiserum injection

We closely analyzed the grade progression from bite in relation to the time (Fig. 2). In the group that received the antiserum within 14 h (Fig. 2A), the grade progressed very rapidly before the antiserum injection. However, after the

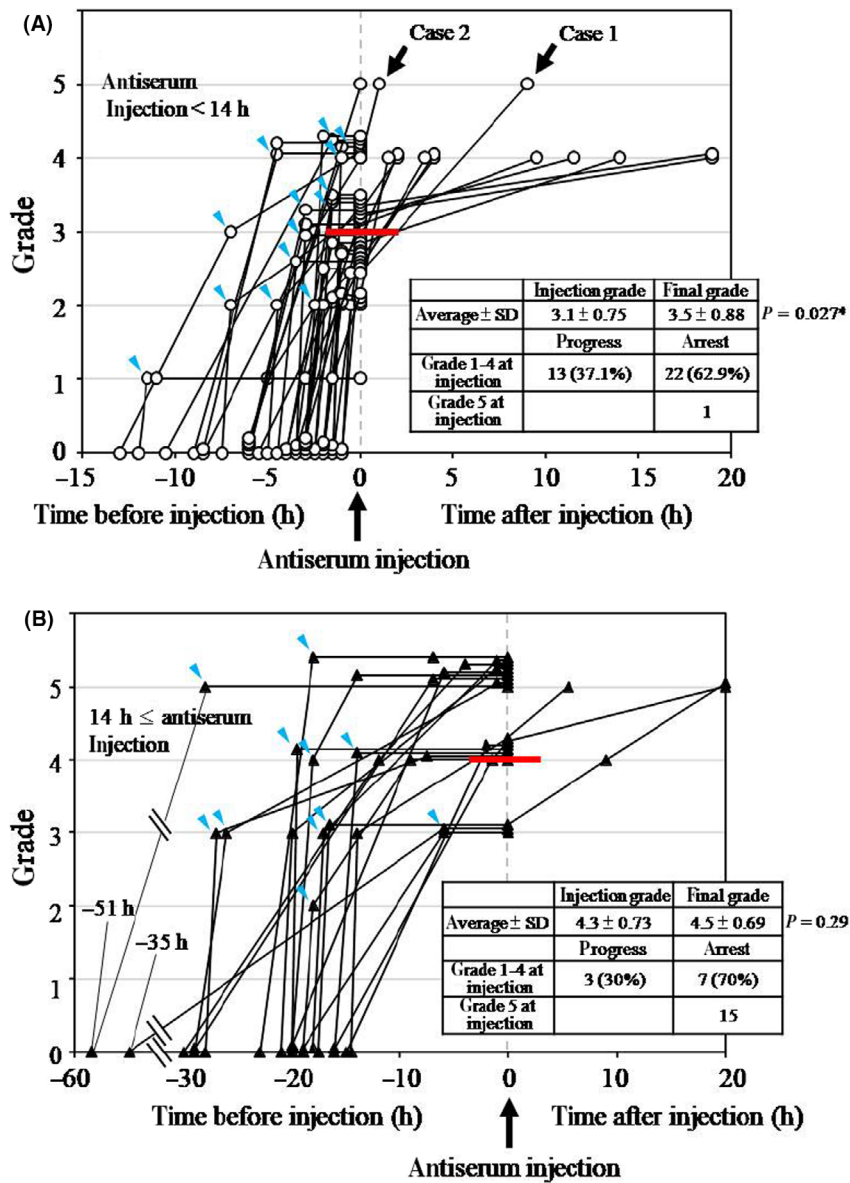


Fig. 2. Grade progression in relation to the time and antiserum injection following mamushi bite. A, Cases that received antiserum within 14 h from the bite. Each line indicates an individual case. The grade before the bite is expressed as grade 0. Y-axis represents grade, and x-axis represents time. Time 0 means the time of antiserum injection as indicated by an arrow in the horizontal bar. By regarding the time of injection as the standard, the time before injection was expressed in minus numerals (h), while the time after injection was expressed in ordinal numerals (h). In cases that presented with grade progression after the antiserum injection, the final grades were plotted. For cases that presented with arrest of the grade progression after the injection, the plots after the injection are not shown. Median injection grade is indicated by a red bar. Time of arrival at the final hospital in representative cases is indicated by blue arrowheads. Cases 1 and 2 are indicated by arrows in the panel. The average injection grade and final grade ± standard deviation and the numbers of cases that showed progression and arrest of the grade after the antiserum injection are shown in the table. The *P*-value is the result of a comparison between the average injection and final grades by the *U*-test. *Statistically significant. B, Cases that received antiserum over 14 h after the bite. The grade progression and statistics are expressed as in (A). Actual onset time is indicated in two cases.

injection, 62.9% of the cases showed arrest of the grade progression (Fig. 2A, table). The median injection grade was 3.0. In contrast, in the group that received antiserum beyond 14 h (Fig. 2B), 70% showed arrest of the grade progression after the antiserum injection, but in 60% of cases, the grades had been arrested for more than 6 h. This shows a marked contrast with the other group in which no cases showed arrest of the grade progression for over 6 h. The median injection grade was 4.0, which was significantly greater than that in the early treatment group ($P = 0.000095$, U -test). The natural trend in the grade indicated quite a rapid progression during the initial 5 h (median grade 2 at initial 2 h, and grade 3 at 2–5 h) after the mamushi bite with most cases reaching grade 4 within 10–20 h (Fig. S1).

Two severe cases with an injection grade below 4 received antiserum within 14 h (Figs 1C and 2A). The clinical summaries of these cases are shown in Table 3. These cases were bitten on the hand/finger, and the maximal swelling extended beyond the shoulder joint. Case 2 developed wheal formation and a decrease in blood pressure during antiserum injection. This case was diagnosed with anaphylaxis, so antiserum injection was terminated at 1/5 volume. Neither renal failure nor DIC was observed in either case.

DISCUSSION

THE PRESENT STUDY clarified that delayed antiserum injection is more frequent in severe cases than in non-severe cases, and 14 h was calculated as the favorable cut-off time that distinguishes severe and non-severe groups. When taking the maximal CK levels into account, because the CK levels are related to disease severity,^{7,8} the maximal CK level in the cases receiving antiserum beyond 14 h was significantly higher than in those receiving it within 14 h (median 7,697 IU/L vs. 203 IU/L, $P = 0.00013$) (Fig. S2). As a result, the elevation of the maximum CK level due to a delayed antiserum injection strongly supports the established cut-off time.

Makino *et al.*⁶ previously compared mamushi bite treatment with and without antiserum and showed that the frequency of general symptoms was markedly higher in antiserum non-injected cases than in antiserum injected cases (65% vs. 13.8%, respectively). This study also indicated that antiserum injection beyond 3 h after a bite frequently resulted in the appearance of general symptoms. The antiserum injection in this report tended to occur in a very early time period (many cases within 2 h), with only a few cases receiving injection beyond 3 h. In contrast, in the present study, over 70% of cases received antiserum injection beyond 3 h. This deviation of the cases might have

Table 3. Description of two cases that received antiserum early but progressed to grade 5

Case	Age	Sex	History	Bitten in	Arrival (h from bite)	Initial grade	Antiserum injection (h from bite)	Injection grade	Swelling	Maximal CK level (IU/L)	Clinical course	Hospital day
1	42	M	None	Hand	2.5	3	3	3	Anterior chest	177	Swelling gradually ameliorated without any problem in general condition	11
2	78	F	Extraterine pregnancy, appendicitis (48 years ago)	Finger	5	4	6	4	Shoulder joint	19,621	Wheal formation during antiserum injection, and the injection was terminated at 1/5 volume. Blood pressure fell later. Swelling peaked out on the 3rd day. Neither renal failure nor DIC was seen	8

resulted in the differences in the cut-off time between this study and the previous report.

Among cases that received the antiserum within 14 h, 35 had an injection grade of 1–4. Of these 35 cases, 30 cases (85.7%) had been bitten within 6 h and showed rapid grade progression before the antiserum injection (Fig. 2A), and 22 (62.9%) showed arrest of progression after antiserum injection. Because the natural trend in the grade indicates quite a rapid progression during an early time period after the mamushi bite (Fig. S1), grade progression in a substantial number of these 35 cases would have been expected to continue during this time period. Therefore, the grade progression in a substantial number of cases appears to have been arrested because of the antiserum injection.

Two cases showed progression to grade 5 despite early antiserum injection (Figs. 1C and 2A, Table 3). There were no critical underlying diseases or any characteristic events in Case 1, so the cause of progression to grade 5 was unclear. In contrast, in Case 2, because of anaphylaxis that developed at the beginning of the antiserum injection, the injection was terminated at 1/5 volume. Therefore, insufficient antiserum injection might have caused the grade to progress to 5.

In the group that received antiserum injection beyond 14 h, the injection grade had already been 5 in nine of the 19 cases (47.4%). Among the other 10 cases with an injection grade 1–4, seven (70.0%) showed arrest of grade progression, and in six of these seven cases (85.7%), the injection time was extremely delayed (average injection time, 21.7 ± 7.2 h). Furthermore, the grade progression of these cases had already been arrested for over 6 h before the injection. According to the natural trend in grade progression (Fig. S1), the progression becomes very slow or is arrested beyond 20 h from the bite. It is possible that the progression had already been spontaneously arrested before antiserum injection. The median and average injection grade of the 10 cases with an injection grade 1–4 were 4 and 3.7 ± 0.48 , respectively. The grade of these cases had thus considerably progressed by the time of injection.

CONCLUSION

IN THE PRESENT study, a preferable cut-off time for antiserum injection that brought a non-severe clinical course was determined to be 14 h. We expect the findings in the present study to serve as a statistical basis for avoiding delayed antiserum injection and hope that these clinical indices are revised following the accumulation of more cases.

DISCLOSURE

Approval of the research protocol: The ethics committee review board of Oita City Medical Association's Almeida Memorial Hospital and Oita Prefectural Hospital, which were organized according to the ethical guidelines for medical and health research involving human subjects developed by the Ministry of Health, Labor, and Welfare of Japan, approved this retrospective study (approval no. 161 and 2-10, respectively).

Informed consent: N/A.

Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

Conflict of interest: None.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Natural trends in grades during the pretreatment period.

Fig. S2. Comparison of maximal creatine kinase (CK) levels between a group that received the antiserum within 14 h and a group that received it beyond 14 h.