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Review Article

Mamushi (Gloydius blomhoffii) Snake Bites in Japan –Current Problems and Clues to a Solution

Osamu Okamoto[1]*, Rui Suzuki[2], Manami Kusatsu[2], Ryuta Nakashima[3], Nobuhiro Inagaki[3], Yoshitaka Kai[4], and Hiroyuki Hashimoto[2]

[1]*Department of Dermatology, Oita City Medical Association's Almeida Memorial Hospital, Japan*

[2]*Department of Plastic Surgery unit, Oita City Medical Association's Almeida Memorial Hospital, Japan*

[3]*Department of Emergency Medicine, Oita City Medical Association's Almeida Memorial Hospital, Japan*

[4]*Department of Dermatology, National Hospital Organization Beppu Medical Center, Japan*

***Corresponding author**

Osamu Okamoto, Department of Dermatology, Oita City Medical Association's Almeida Memorial Hospital, Japan, Tel: 81 97 569 3121; Email: ookamoto@oita-u.ac.jp

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Abstract

Venomous snakes of the genus Gloydius are distributed in eastern Asia. Bites from one species, known as Japanese Mamushi, Gloydius Blomhoffii, are common in Japan. Some patients develop severe symptom, represented by rhabdomyolysis and acute renal failure, and in extreme cases, death has resulted mainly due to intestinal bleeding/necrosis and perforating peritonitis. The mortality rate is estimated to be about 1 death/300 bites. The lethal cases presented with severe abdominal symptoms, including melena and ileus, and the severe cases including the lethal cases present with higher creatinine kinase values and white blood cell counts. Therefore, it was found that these are reliable indicators predicting the severity of envenomation. The severe and non-severe cases can often be distinguished by the rate of elevation of these laboratory values. At present, mamushi-specific antivenom is recognized as the sole effective medicine against a mamushi bite; however, the clinical results might fail to meet expectations. In this review, we discuss problems associated with treating cases of mamushi bite, our recent trials, and perspectives regarding solutions to the clinical problems encountered.

INTRODUCTION

There are several venomous terrestrial snakes in Japan with two species considered of extreme medical significance. One of these, Habu (*Protobothrops flavoviridis*) is restricted to the south-western islands, while another, Japanese Mamushi (*Gloydius blomhoffii*, formerly *Agkistrodon blomhoffii*) is distributed widely in Japan, except for the south-western islands [1,2]. As one might expect, with its much wider distribution, the frequency of mamushi bites significantly exceeds the other species, making it a common event in Japan, with an estimated 3,000 people being bitten and 10 dying from the bite [3]. Mamushi-bitten patients are often treated with mamushi-specific antivenom serum, which is the only medicine that can theoretically neutralize the mamushi venom, however the clinical effectiveness of this serum is obscure. In addition, the causes of the envenomated victim's death are poorly known. Furthermore, to date, there have not been available quantitative indicators that reflect the severity of a mamushi bite.

In this review, we describe the clinical progression in cases that resulted in death and discuss some bite severity indicators that can help physicians recognize and predict the clinical course and outcome. We also highlight the current problems associated with treating bitten patients and discuss ways to solve these problems.

GENERAL DESCRIPTION OF MAMUSHI SPECIES

Mamushi is a viper that is widely distributed in Japan [1]. Its taxonomical position is as follows:

Family: Viperidae, Subfamily: Crotalinae, Genus: Gloydius, Species: blomhoffii

Some other closely related Gloydius species are also distributed in the eastern areas of Asia and these include *G. tsushimaensis, G. brevicaudus, G. ussuriensis* [4]. The Japanese Mamushi is a pit viper with adults between 40-80 cm in length, usually pale to dark brown in color with dark-edged blotches (for photographs, see [5]). It is mainly active at night during the summer, so most of bites occur at that time and early morning. Mamushi inhabits the wet, weedy areas typically around rice paddies. It does not jump, and only bites when victim draws near, so unsuspecting farmers are commonly bitten when maintaining their rice paddies and farms. Seventy percent of patients are bitten on the extremities [6].

CHARACTERISTICS OF MAMUSHI VENOM

Like in other vipers, mamushi venom is comprised of a mixture of many different enzymes and non-enzymatic components (Table 1) [7,8]. These include phospholipase A2, hyaluronidase, L-amino acid oxidase, phosphodiesterase, and 5' nucleotidase, which are reported to be the most common components of the venoms found in viper species [7,8]. Phospholipase A2 in Clotarinae subfamily has molecular weight between 11-31 kDa [8]. This enzyme in habu venom is supposed to cause rhabdomyolysis, hemorrhaging, and local swelling [8], and the toxic effects are similar in another Crotalinae member [9]. Hyaluronidase does not have poisonous action by itself, but it breaks down mucopolysaccharides, facilitating diffusion of the venom components into the connective tissue. Particularly, hyaluronidase enhances hemorrhaging effects of hemorrhagic factors (HR) mentioned below [7]. L-amino acid oxidase of venomous snake is quite potent among L-amino acid oxidase homologues found in other animals. It is a 60 kDa acidic glycoprotein and it induces apoptosis of the vascular endothelial cells, and inhibits factor IX function and platelet aggregation [10,11]. These biochemical functions of the L-amino acid oxidase would contribute to the subcutaneous hemorrhage usually seen in mamushi-bitten patients. Phosphodiesterase, and 5' nucleotidase are nucleic acid-degrading enzymes and it is supposed that they are potentially cytotoxic. However, their harmful in vivo effects have not ever been concerned.

Aside from these common components of venoms, arginine ester hydrolase is highlighted in all Gloydius species as well as in the Crotalinae subfamily. Arginine ester hydrolase comprises at least three enzymes: "bradykinin releasing enzyme", "clotting enzyme", and "permeability increasing enzyme" which were named in the original publication [12]. These three enzymes have molecular weight of 30 kDa [8]. The bradykinin releasing enzyme releases bradykinin from plasma kininogen and thus induces hypotension. This enzyme was later identified as mamushi salivary kallikrein [13]. The clotting enzyme induces blood coagulation by converting fibrinogen to fibrin; and the permeability increasing enzyme induces vascular hyperpermeability, resulting in swelling [7, 12]. Further characterization of these Japanese mamushi enzymes could not be found in the following publications. However, amino acid sequence of bradykinin releasing enzyme homologue in *Gloydius caliginosus* (Korean mamushi) has been determined [14]. Also, molecular cloning of a subtype of capillary permeability-increasing enzyme homologue in the same snake species has been done [15].

Mamushi venom contains two hemorrhagic factors: HR-1 and HR-2 [7,16,17]. A series of investigations on mamushi venom by Suzuki et al. stressed that the lethal activity of mamushi venom is essentially due to these factors [7,16,17]. Both are acidic glycoproteins; the molecular weight of the HR-1 is estimated to be 85 kDa [16,18], and that of HR-2 95 kDa [17]. When these hemorrhagic factors were intraperitoneally injected into mice and the deaths within 24 h were assessed, the LD50 of HR-1 and HR-2 was estimated to be 0.36 and 4.96 mg/kg, respectively [16]. Thus, the lethal activity of HR-1 is about 10 times as potent as that of HR-2. The HR-1 is therefore likely the main lethal factor of mamushi venom. The LD50 of crude mamushi venom was estimated to be 1.32 mg/kg in the same study [16]. In contrast, in humans, deaths within 24 h have not been known, and lethal outcomes generally take more than several days to develop [19,20]. Therefore, the actual LD50 of the venom in humans is expected to be much lower than that reported in mice.

When intravenously injected into mice, HR-1 induces drastic intestinal hemorrhaging, whereas subcutaneous hemorrhaging is mild [7]. The intestinal hemorrhaging closely resembles that seen in human lethal cases, as described in the section "Causes of deaths by mamushi bite". In contrast, the hemorrhaging induced by HR-2 is largely subcutaneous and intramuscular [7]. The hemorrhagic activity of HR-1 is estimated to be 30-50 times as potent as that of HR-2 [7,16]. Chelators such as ethylenediaminetetraacetic acid (EDTA) and cysteine, which bind and deplete the environmental divalent cations, cancel the lethal activities of HR-1, HR-2, and crude mamushi venom [16,21]. Importantly, the inhibition of these hemorrhagic factors is irreversible. Thus, these hemorrhagic factors essentially require divalent cations to maintain their functional conformation. This observation may be useful for treating patients suffering from mamushi bites.

Apart from the components above, mamushi venom contains a platelet aggregation protein, mamushigin [22]. It is a heterodimeric 30 kDa protein and it can bind platelet glycoprotein-I, but it can also directly aggregate platelets [22]. The unique function of the mamushigin is believed to cause thrombocytopenia via platelet aggregation [23,24]. However, this symptom is not often seen in mamushi-bitten human victims. Here, mamushigin induces platelet aggregation whereas L-amino acid oxidase inhibits platelet aggregation, thus their functions are opposite. Considering from the frequency of the thrombocytopenia and subcutaneous hemorrhaging, it is supposed that the effect of the L-amino oxidase is normally predominant, and the effects of mamushigin appear in a special condition, such as direct intravascular injection of the venom, as described below. Neurotoxins (α- and β-toxins) are also found in mamushi venom. Alpha-toxin binds postsynaptic acetylcholine (ACh) receptor and blocks the nerve conduction, whereas β-toxin, by disrupting presynaptic ACh release, blocks the conduction [25,26]. These neurotoxins are thought to contribute to oculomotor paralysis.

CLINICAL SYMPTOMS OF MAMUSHI BITE

Because a mamushi possesses two functioning fangs, the number of bite wounds is typically two. However, sometimes a bite occurs incompletely or several times to the one site, so the number of bite wounds can vary. A diagnosis of mamushi bite is therefore best achieved by observing the clinical symptoms. The first symptom is local swelling around the affected site (Figure 1A). This swelling usually accompanies hemorrhaging in the deeper part. The hemorrhaging and the swelling can be explained by the functions of L-amino acid oxidase [10,11] and permeability increasing enzyme [12], respectively. A grading system for swollen areas has been proposed and is adapted for the treatment of mamushi bites (Figure 1B) [27]. In many cases, the swelling is restricted to the extremities; therefore, if the swelling is extreme or the muscle damage is severe, compartment syndrome can happen. The swelling can sometimes proceed from

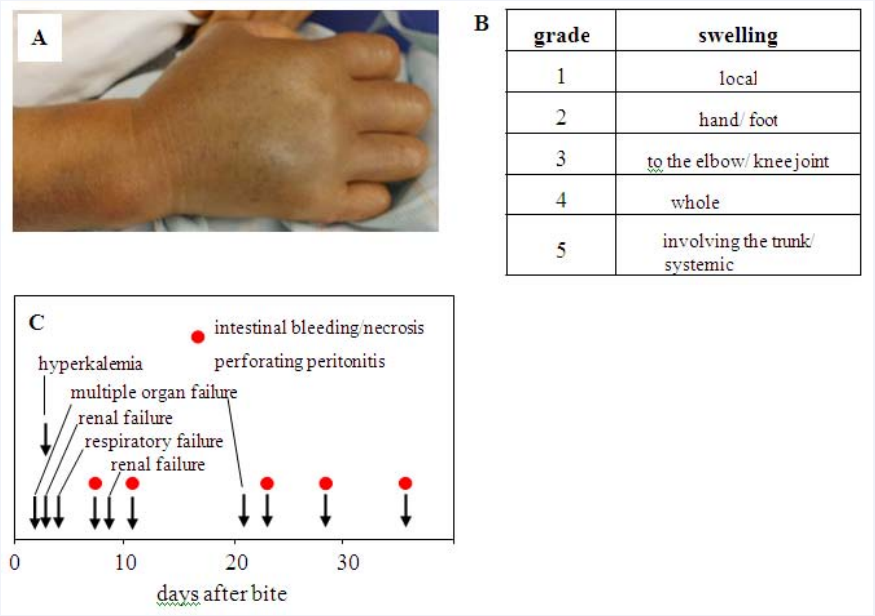

Figure 1 A: Swelling of the hand after a mamushi bite. The fingers are commonly fixed in a claw position because of the swelling. The purplish color reflects the deep ecchymosis. B: Grades of swelling after mamushi bite (originally published by Sakio et al. [27]). C: Causes of deaths due to mamushi bite in relation to the time after bites. Each lethal case is indicated by an arrow. The principal cause of each death is indicated in the panel. Panels A and B were quoted from Ref. [29].

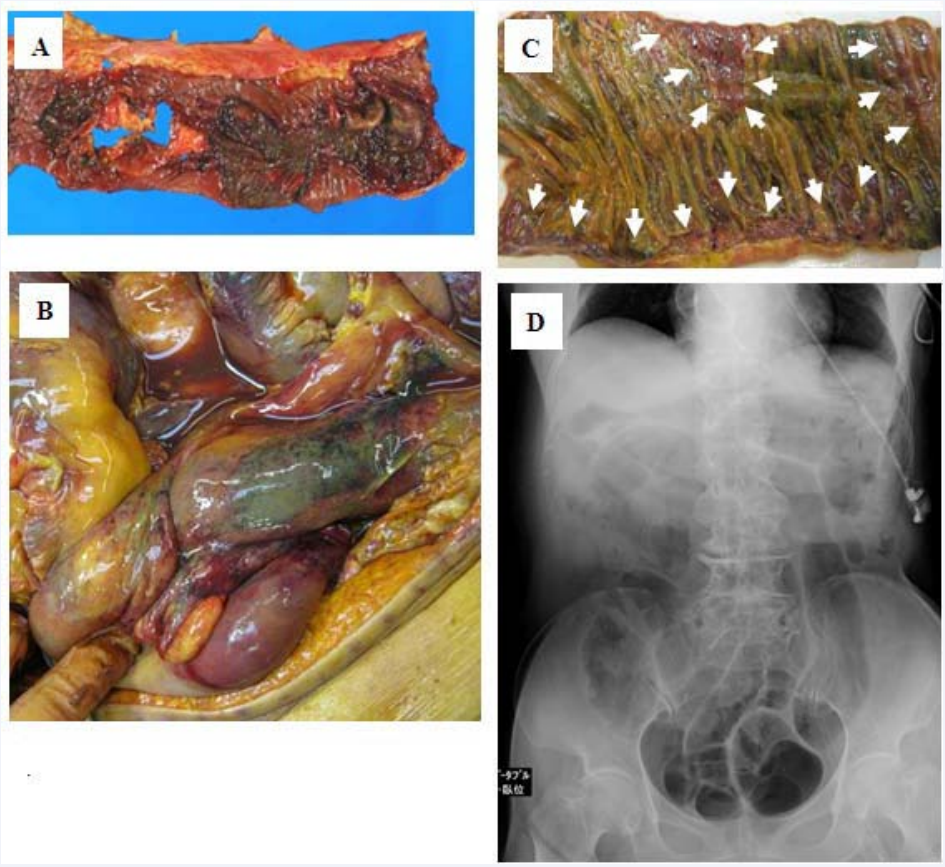

Figure 2 A: Perforation of the sigmoid colon seen during an operation. B: Bleeding necrosis of the intestines seen at an autopsy. C: Loss of epithelium (arrows) seen in the intestine during an autopsy. D: Expanded bowels due to paralytic ileus on abdominal X-ray on the second day after the bite. Fig. 2 was quoted from Ref. [29] with some modifications.

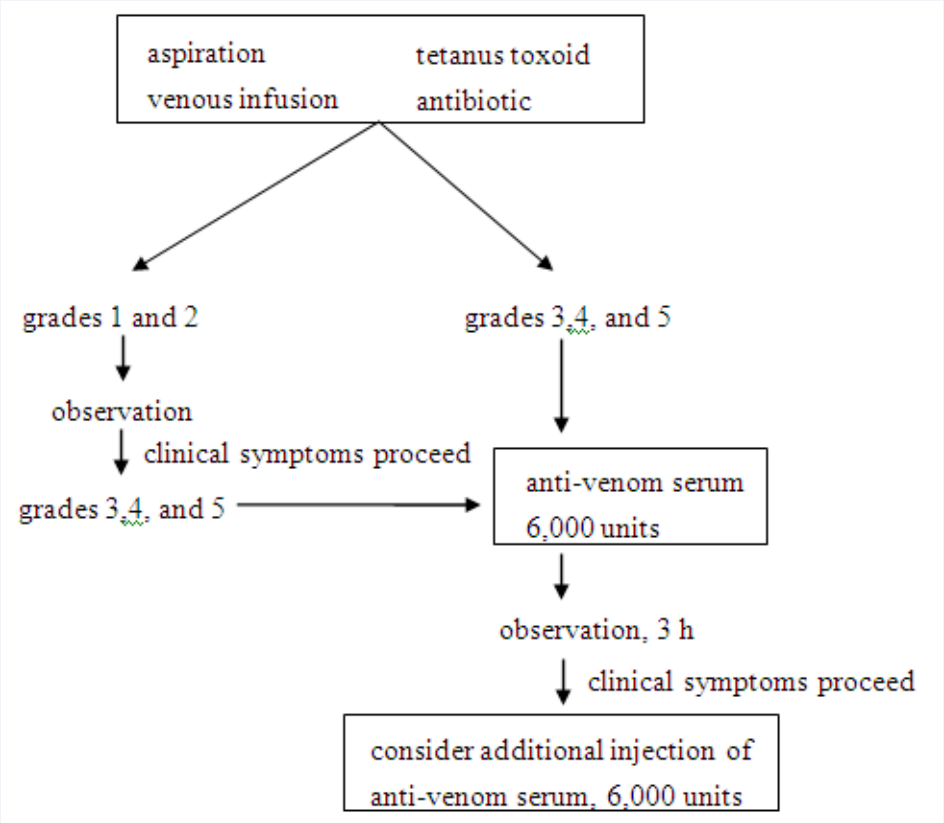

Figure 3 Therapeutic protocol for mamushi bites. These findings were based on a previous publication [27], with some modification in the expression. The figure was quoted from Ref. [43].

the extremities to the trunk. In such cases, the clinical course tends to be severe, with accompanying general symptoms.

The principal general symptom is acute renal failure, which is usually associated with severe rhabdomyolysis [28]. The rhabdomyolysis is reflected by serum creatine kinase (CK) as well as myoglobin levels, and the CK levels are commonly monitored. The responsible venom component for the renal failure as well as rhabdomyolysis has not been clearly stated in the previous literature. Acute renal failure in mamushi bite is supposed to happen because of the obstruction of glomerular capillaries by excess myoglobin, and a decrease of renal blood flow due to excess swelling [1]. Therefore, components that induce muscle damage and swelling, such as phospholipase A2 and permeability increasing enzyme as appeared in the previous section, would be the candidates for the renal failure. Rarely, if the rhabdomyolysis is severe, a large amount of potassium is released into the blood, sometimes leading to cardiac arrest because of acute hyperkalemia. Acute liver necrosis can also occur, as we previously reported [29]. When bradykinin is released by arginine ester hydrolase [7,8], hypotension can be induced, and when the chest wall is severely affected by swelling, even respiratory arrest can occur. Acute bleeding diathesis devoid of disseminated intravascular coagulation syndrome can happen due to platelet aggregation induced by the venom. This phenomenon is believed to be caused by the action of mamushigin in the venom injected directly into the vessels [23,24]. This special type of mamushi bite is called the thrombocytopenia type. Oculomotor paralysis sometimes appears and is included among the general symptoms [27]. This symptom is due to neurotoxins (α- and β-toxins) that are also present in the mamushi venom [26]. However, it usually disappears within a couple of weeks, and is not always associated with a severe general condition [30]; therefore, oculomotor paralysis itself is an exceptionally non-severe general symptom

CAUSES OF DEATHS BY MAMUSHI BITE

Ten people a year are estimated to die from mamushi bites in Japan. We referred the lethal cases in publications and analyzed the causes of death in relation to the time after bite. Based on the publications [19,20,29,31-38], the causes of death can be roughly classified into 2 timeframe groups: within 5 days, and over 20 days (Figure 1C). The causes of deaths that occurred within 5 days are varied, including hyperkalemia, respiratory failure, renal failure [19,20,35,38]. In contrast, the cause of deaths that occurred over 20 days after a bite was predominated by intestinal bleeding/necrosis and perforating peritonitis [29,32,33] (Figure 2A-C). Because HR-injected mice demonstrate intestinal bleeding/necrosis [7] identical to that seen in human, it is evident that the HR induces these severe findings. In three of these cases, the victims presented with ileus and melena during the acute period [29,32,33] (Figure 2D). Therefore, a combination of the above symptoms is regarded here as signs of a lethal outcome. Most of the lethal cases demonstrate the renal failure in an acute period, therefore the victims who survived the renal failure etc. demonstrate intestinal bleeding/necrosis and perforating

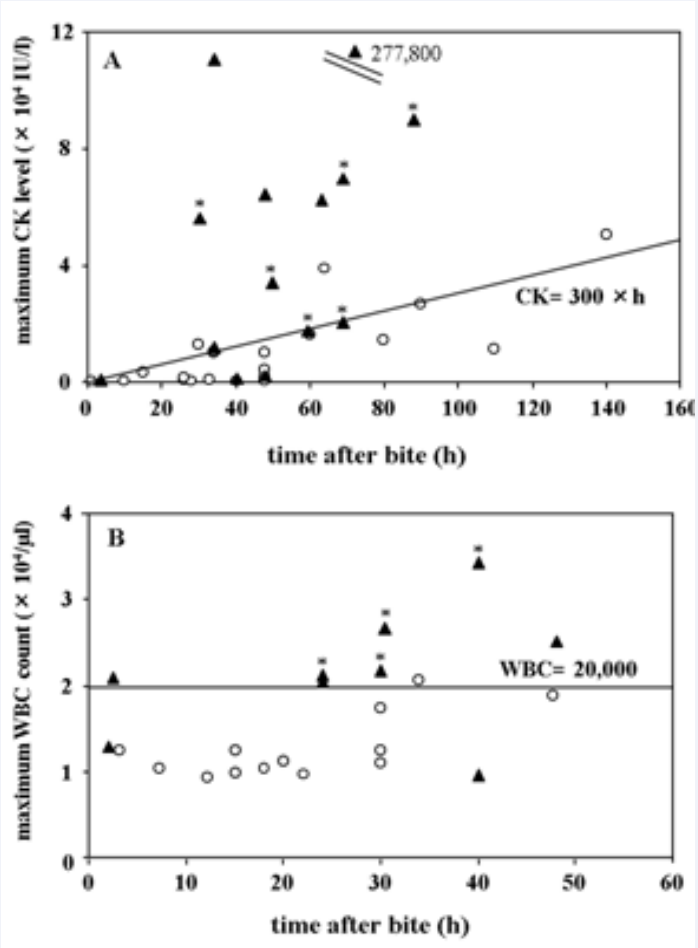

Figure 4 Comparisons of severe and non-severe cases by the maximum laboratory values-time after bite relationships. A: Maximum CK levels. White circles indicate non-severe cases and black triangles the severe cases. The horizontal line indicates the time after the bite, the vertical line the maximum CK levels. The line Y=300X distinguishes many of the non-severe cases from the severe cases. B: Maximum WBC counts. White circles and black triangles indicate the non-severe and the severe cases, respectively. The horizontal line indicates the time after the bite, the vertical line the maximum WBC counts. The line Y=20,000 distinguishes many of the non-severe cases from the severe cases. In panels A and B, asterisks indicate the lethal cases. The panels were quoted from Ref. [49] with some modifications.

peritonitis in the later period, resulting in a lethal outcome. The renal failure is understood as a possible cause of death however, the intestinal bleeding/necrosis and perforating peritonitis is not widely known as the causes of delayed lethal outcome. This may be because the lethal cases have rarely been reported in spite of its frequency (10 deaths/year in Japan). In principle, injection of larger amount of venom by a deep bite should be important for suspecting a severe outcome. However, it is not possible to estimate the amount of the injected venom and there is no way of predicting the severe outcome by evaluating the bitten situation. The trials of prediction of the severe outcome are discussed in a section "Trials for predicting the clinical course of mamushi bite".

KINETICS OF MAMUSHI VENOM IN VIVO

Although how mamushi venom behaves after injection into human victims remains unclear, experimental animal models have provided useful findings [39]. When 1.5 mg of mamushi venom was injected into the muscle of rats, the serum mamushi venom concentration peaked at 760 ng/ml 3 h after injection. About 20% of the venom retained in the serum at 48 h after the injection. Only about 10% of the venom remained at the injected site at 1 h, eventually decreasing to the baseline level at 6 h. Taken together, these data indicate that about 90% of the intramuscularly injected mamushi venom swiftly disappears from the injected site and migrates into the blood. In the serum, the venom concentration peaks at 3 h and maintains its concentration for a long time.

TREATMENTS OF MAMUSHI BITE

The appropriate treatment of mamushi bite has not been established, although a system for grading mamushi bites and a treatment flowchart has been proposed (Figure 3) [27]. In the original flowchart, cepharanthine and incision of the bitten area were included in the initial treatment protocol, but the clinical effects of these treatments have been disputed [40]. Thus, the initial treatments may vary; however, it is invariably accepted that mamushi specific antivenom is administered for bites of grades 3 severity or worse [27,41].

In experimental animal models, where intravenous antivenom

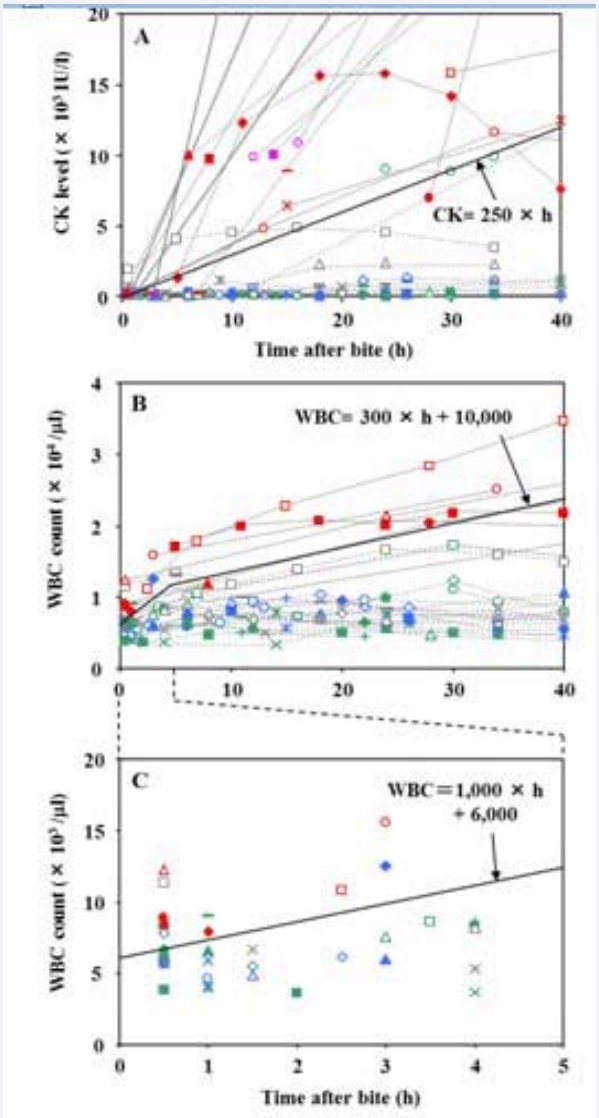

Figure 5 Comparisons of the rates of increase in the laboratory values between severe and non-severe cases. A: Trends in the CK levels in relation to the time after bites. Red and magenta symbols indicate the severe cases, and blue, green and gray symbols indicate the non-severe cases. The cut-off line that distinguishes the non-severe cases from the severe cases is drawn with a black solid line, and its equation is shown in the panel. The horizontal line indicates the time after the bite, the vertical line the CK levels. B,C: Trends in the WBC counts in relation to the time after bites. B: The trends until 40 h from the bite and C: until 5 h from the bite. Red and magenta symbols indicate the severe cases, and blue, green and gray symbols indicate the non-severe cases. The cut-off line that distinguishes the non-severe cases from the severe cases is drawn with a black solid line, and the equations are shown in the panels. The horizontal line indicates the time after the bite, the vertical line the WBC counts. The panels were quoted from Ref. [43] with some modifications.

was given after the intramuscular injection of mamushi venom, the detectable serum mamushi venom concentration was suppressed to the baseline level after 1 h and remained almost the same thereafter. Similarly, the residual mamushi venom at the injection site decreased to the baseline level at 6 h [39,42]. In the experimental setting, it is evident that the antivenom suppresses the concentration of detectable venom, although the antivenom used in those previous studies was a different product from that being used presently (Figure 3).

At present, antivenom is commercially supplied from another pharmaceutical company and exclusively used in the clinical field in Japan. One remarkable effect of the antivenom is the recovery of the platelet count in thrombocytopenia type mamushi bites [24,44]. However, in most other cases, the clinical effectiveness of the mamushi specific antivenom is obscure. A recent multicenter trial only demonstrated a shorter hospital stay in the patients administered the antivenom [45]. The serum CK levels tended to be lower in the antivenom-treated group, but no other significant difference was found. Another study came to a similar conclusion [6].

In most previous reports, the timing of antivenom injection after the bite was not noted, although in one study concerning the timing of antivenom administration in 114 viper bites found a difference in the frequency of general symptoms between patients receiving the early (within 3 h) and late (more than 3 h) administration of antivenom [46]. The early administration of antivenom may therefore partially prevent the clinical course from progressing to a severe one. However, information on the timing of antivenom administration is currently insufficient, suggesting a large-scale assessment of this is required.

At present, antivenom is the only medicine that can theoretically neutralize the mamushi venom, but the beneficial clinical effects of this may be generally overestimated. Therefore delayed antivenom administration has come to be recognized as a serious problem, with such delays sometimes leading to court cases of malpractice [47].

Of serious concern is the incidence of anaphylaxis in some recipients of antivenom. Because it is produced from horse serum, it contains heterologous proteins that can potentially cause allergic reactions, including anaphylaxis as the most severe manifestation of these. One report described the presence of IgG-type anti-horse serum antibodies in patients several weeks after horse antivenom serum had been administered [48]. The report also stated that IgE-type antibodies were detected in about 10 % of cases, with these anti-horse serum antibodies still positive even several years after the administration [48]. However, among our previously reported cases, none presented with anaphylaxis [43]. Furthermore, in a recent report comprising 81 cases, although systemic steroids had already been given, allergic reactions were reported as wheal formation only, and no cases presented with anaphylaxis [6]. The frequency of antivenom-induced anaphylaxis is therefore likely low. In addition, premedication with systemic steroids or antihistamine reagents prior to antivenom injection might prevent the occurrence of anaphylaxis in most anti-horse serum antibody-positive cases [1]. In conclusion, physicians do not have to hesitate to administer the antivenom, but it should always be administered in appropriate clinical settings.

TRIALS FOR PREDICTING THE CLINICAL COURSE OF MAMUSHI BITE

A troublesome issue with mamushi bite is that no clinical indices to determine a life-threatening outcome have been

Table 1: Principal mamushi venom components and the toxicity.

Prinicipal enzymes/ components	Toxicity
phospholipase A2	rhabdomyolysis, hemorrhaging, swelling
hyaluronidase	facilitation of venom diffusion by degradation of mucopolysacchrides
L-amino acid oxidase	inhibition of blood coagulation. Inhibition of platelet aggregation
phosphoesterases	nucleic acid degradation
5' nucleotidase	nucleic acid degradation
arginine ester hydrolase	
bradykinin releasing enzyme	hypotension due to bradykinin release
clotting enzyme	blood coagulation
permeability increasing enzyme	swelling due to capillary hyperpermeability
hemorrhagic factor-1 (HR-1)	intestinal bleeding/necrosis
hemorrhagic factor-2 (HR-2)	skin bleeding
mamushigin	thrombocytopenia due to platelet aggregation
neurotoxins (α-and β-toxins)	oculomotor paralysis

established. If we can predict the severity of a mamushi bite, delays in the administration of antivenom can be avoided, and appropriate therapeutic preparations can be made in advance. In our attempts to establish such an index, we first focused on the maximum CK levels and white blood cell (WBC) counts. Assessments were performed by dividing patients into severe and non-severe cases. Severe cases were defined as those who demonstrated life-threatening clinical manifestations, such as renal failure, DIC, and respiratory insufficiency (for details, see [43,49]). We found that the cases that presented with higher maximum CK values in relation to the time after the bites tended to be severe, and maximum WBC counts over 20,000 / μl also showed severe clinical manifestations, including a high non-survival rate [49] (Figure 4). However, because the study was based on trends in the maximum laboratory values, the clinical course could have already progressed before making our prediction. Therefore, in a subsequent publication, we focused on the rate of increase in the CK value and WBC count in relation to the time after the bite to enable a real-time prediction of the clinical course [43]. As shown in Figure 5, the rate of increase in both laboratory values differed between the severe and non-severe cases, and these cases were able to be distinguished based on the equations shown in the figure. These findings indicate that predicting severe cases is possible as early as several hours after the bite has occurred.

PERSPECTIVES

Preventing deaths due to mamushi venom is a top priority. Therefore, the preparation of neutralizing antibodies against the lethal factors is crucial. As stated above, discrepancies in the antivenom effects between experimental and clinical settings remain a problem. Experiments have demonstrated the rapid loss of detection of mamushi venom on an ELISA after the intravenous injection of antivenom [42]. However, whether the majority of the mamushi venom was really neutralized and no longer capable of inducing harmful effects on the victims remains unclear. To our knowledge, whether the presently available antivenom neutralizes the crucial components of mamushi venom, including HR-1 and HR-2, has not been confirmed. Therefore, the assessment of the neutralizing ability of the antivenom using experimental animals is needed.

Another important issue is the production of recombinant anti-mamushi venom human antibodies. Efforts to this end are already underway in the field of anti-habu venom antibodies [50]. The local administration of chelators at the early phase of a mamushi bite may also be effective, as they cause HR-1 to lose its lethal activity irreversibly [16]. Because cysteine is an authentic amino acid and a chelator, this would be a good candidate. The monitoring of serum and tissue for mamushi venom concentration may aid in the evaluation of the venom activity as well as the correct diagnosis of mamushi bites. A detection system using an ELISA has been developed for experimental animal models [39,42]; therefore, the adaptation of this system for the clinical field is expected.

CONCLUSION

The initial treatments of mamushi snakebite patients still do not obtain general agreement therefore, by assessing their clinical usefulness one by one, establishment of a standardized therapeutic protocol is expected. In addition, a theoretical background of the effectiveness of presently available mamushi specific antivenom is needed. Although the present information about mamushi venom largely depends on research in the 1960's and 1970's, by reevaluating and revising this knowledge, strategies for treating the mamushi snakebite would be improved.

REFERENCES

1. Sakai A, Moriguchi H, Toriba M. Guide of venomous snake bites for field workers. Bulletin Herpetol Soc Japan. 2002; 75-92.
2. Sakai A. Snakes snake venoms. J Public Health Pract. 2010; 74: 377-381.
3. Mamushi SA, Habu, Yamakagashi. Clinical Efficacy of Antivenom and Cepharanthine for the Treatment of Mamushi (Gloydius blomhoffii) Bites in Tertiary Care C. Med Clin Japan. 2001; 27: 1911-1915.
4. McDiarmid RW, Campbell JA, Toure TA. Snake Species of the World: A Taxonomic and Geographic Reference, Volume 1. Washington, D.C., Herpetologists' League. 1999.
5. Hifumi T, Sakai A, Kondo Y, Yamamoto A, Morine N, Ato M, et al. Venomous snake bites:clinical diagnosis and treatment. J Intensive Care. 2015; 3: 16.
6. Kimura N, Okabe N, Futamura-Nakagawa R, Koda F, Izumo A. Furue M. Clinical study of 81 cases of "Mamushi" viper bite during the past 11 years. Nishi-Nihon J Dermatol. 2015; 77: 584-588.
7. Suzuki T. Studies on snake venom enzymes, centering around Agkistrodon halys blomhoffii venom. Snake. 1970; 2: 75-94.
8. Chinzei H. Viper venom and the related matters. Kagaku to Seibutsu. 1987; 25: 130-140.
9. Gutiérrez JM, Lomonte B. Phospholipase A2 myotoxins from Bothrops snake venoms. Toxicon. 1995; 33: 1405-1424.

10. Takatsuka H, Sakurai Y, Yoshioka A, Kokubo T, Usami Y, Suzuki M, et al. Molecular characterization of L-amino acid oxidase from Agkistrodon halys blomhoffii with special reference to platelet aggregation. Biochim Biophys Acta. 2001; 1544: 267-277.

11. Sakurai Y, Shima M, Matsumoto T, Takatsuka H, Nishiya K, Kasuda S, et al. Anticoagulant activity of M-LAO, L-amino acid oxidase purified from Agkistrodon halys blomhoffii, through selective inhibition of factor IX. Biochim Biophys Acta. 2003; 1649: 51-57.

12. Sato T, Iwanaga S, Mizushima Y, Suzuki T. Studies on snake venoms. XV. Separation of arginine ester hydrolase of Agkistrodon halys blomhoffii venom into three enzymatic entities: "bradykinin releasing", "clotting" and "permeability increasing ". J Biochem. 1965; 57: 380-391.

13. Iwanaga S, Suzuki T. Characterization of the bradykinin releasing enzyme in the venom of Agkistrodon halys blomhoffii as salivary kallikrein of the snake. Hoppe Seylers Z Physiol Chem. 1968; 349: 928-929.

14. Oyama E, Fukuda T, Takahashi H. Amino acid sequence of a kinin-releasing enzyme, KR-E-1, from the venom of Agkistrodon caliginosus (Kankoku-mamushi). Toxicon. 2008; 52: 651-654.

15. Hahn BS, Baek K, Kim WS, Lee CS, Chang IL, Kim YS. Molecular cloning of capillary permeability-increasing enzyme-2 from Agkistrodon caliginosus (Korean viper). Toxicon. 1998; 36: 1887-1893.

16. Oshima G, Omori-Sato T, Iwanaga S, Suzuki T. 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.

17. Iwanaga S, Omori T, Oshima G., Suzuki T. Studies on snake venoms. XVI. Demonstration of a proteinase with hemorrhagic activity in the venom of Agkistrodon halys blomhoffii. J Biochem. 1965; 57: 392-401.

18. Nikai T, Oguri E, Kishida M, Sugihara H, Mori N, Tu AT. Reevaluation of hemorrhagic toxin, HR-I, from Agkistrodon halys blomhoffii venom: proof of proteolytic enzyme. Int J Biochem. 1986; 18: 103-108.

19. Yada S, Yamaguchi T, Miyauchi T, Kuratate S, Yogita S. A clinical study of 37 cases of venomous snakebite injuries. J Jpn Soc Clin Surg. 2006; 67: 2788-2791.

20. Akishima S, Takahashi K. Hisakura K. Sakurai J. A case of critical injury by viper bite in a patient who underwent aortic valve replacement. Surgery. 2005; 67: 329-332.

21. Omori T, Iwanaga S, Suzuki T. The relationship between the hemorrhagic and lethal activities of Japanese mamushi (Agkistrodon halys blomhoffii) venom. Toxicon. 1964; 15: 1-4.

22. Sakurai Y, Fujimura Y, Kokubo T, Imamura K, Kawasaki T, Handa M, et al. The cDNA cloning and molecular characterization of a snake venom platelet glycoprotein Ib-binding protein, mamushigin, from Agkistrodon halys blomhoffii venom. Thromb Haemost. 1998; 79: 1199-1207.

23. Mizokami S, Eguchi H, Nakama T. A case of viper bite with thrombocytopenia immediately after injury. Jpn J Clin Dermatol. 2017; 71: 543-547.

24. Okuzawa Y, Nakai N, Katoh N, Suehiro M. A case of mamushi bite with marked thrombocytopenia. Jpn J Clin Dermatol. 2012; 66: 737-740.

25. Nirthanan S, Gwee MC. Three-finger alpha-neurotoxins and the nicotinic acetylcholine receptor, forty years on. J Pharmacol Sci. 2004; 94: 1-17.

26. Yamada M. Oculomotor abnormality due to snake venom. in: Mimura O, editor. Clinical neuro-ophthalmology. Tokyo: Medical Aoi press, 2001; 146-147.

27. Sakio H, Yokoyama K, Uchida T, Oda N, Yamashita S, Miyake K. Mamushi (viper) bite in Kensei General Hospital. J Clin Surg. 1985; 40: 1295-1297.

28. Sakai A. Diagnosis and treatment of snakebite by Mamushi and Yamakagashi. Jpn J Clin Toxicol. 2013; 26: 193-199.

29. Okamoto O, Nakashima R, Yamamoto S, Hashimoto T, Takasaki T, Tokuda H, et al. A lethal case of mamushi (Gloydius blomhoffii) bite: severe bowel symptoms as a lethal sign. Acute Med Surg. 2016; 4: 135-139.

30. Yoshida Y, Kobayashi L, Doshiro A, Ban Y. A case of oculomotor disorder in viper bite envenomation. J Eye. 2004; 21: 1587-1590.

31. Koutoku T, Kaguchi A, Kiyoi T, Nakamura T, Shin Y, Kimura T. A lethal case of mamushi bite. Nishi-Nihon J Dermatol. 2002; 64: 318-321.

32. Oishi M, Okamoto O, Fujiwara S, Kato A, Shimizu F, Sato H, et al. A lethal case of mamushi bite. Jpn J Clin Dermatol. 2008; 62: 407-410.

33. Abe M, Takai K, Tasaki M, Noda K, Shinohara I. A case of viper bite barely treated by the upper arm amputation. Jpn J Acute Med. 2001; 25: 368-370.

34. Fujiwara I, Nomi S, Naitou K, Makino H, Toda S, Nakaji K, et al. A study of 58 cases of viper bite. J Jpn Soc Clin Surg. 1992; 53: 1451-1458.

35. Uematsu M, Sawamura T, Hattori T, Yasufuku M. Review of 29 viper bites -advantage of antivenin therapy-. J Jpn Soc Clin Surg. 1994; 55: 54-60.

36. Kaneko N, Chida A, Okada Y. Initial management of viper bite: A report of two severe cases. J Jpn Soc Emerg Med. 2005; 8: 378-384.

37. Takana K, Yamaguma K, Sato T, Okamoto K, Katsuya H. Fatal multiple organ failure due to mamushi bite. Report of a case. Jpn J Acute Med. 1989; 13: 1867-1870.

38. Kanehisa Y, Taketadzu F, Nagamatsu K. A fatal case of rhabdomyolysis associated with acute renal failure after viper bite. Neurol Med. 1989; 30: 94-96.

39. Watanabe M, Sakai A, Kouda T, Sawai Y. Detection of Agkistrodon b. blomhoffii venom in serum of mice by enzyme-linked immunosorbent assay (ELISA). Snake. 1988; 20: 25-29.

40. Taki K, Ariyoshi K, Sakai A, Ishikawa H, Nakashima K, Endoh Y. Analysis of viper bites by the national survey. J Jpn Soc Emerg Med. 2005; 17: 753-760.

41. Chiba T, Koga H, Kimura N, Murata M, Jinnai S, Suenaga A, et al. Clinical condition and management of 114 mamushi (Gloydius blomhoffii) bites in a general hospital in Japan. Intern Med. 2017

42. Hatsuse M, Sakai A, Sawai Y. Detection and quantitative assay of Agkistrodon h. blomhoffii venom and antivenom by enzyme linked immunosorbent assay (ELISA) with special reference to in vivo effect of the antivenom. Snake. 1990; 22: 20-27.

43. Okamoto O, Sato S, Sakai T, Uehara M, Takenaka R, Ito A, 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.

44. Morita C, Nobuki H, Yanase T. A case of mamushi bite with marked thrombocytopenia and high values of serum CK. Jpn J Clin Dermatol. 2017; 71: 1093-1097.

45. Hifumi T, Yamamoto A, Morokuma K, Okada I, Kiriu N, Ogasawara T, 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.

46. Makino M, Yurugi E, Abe J. A study of 114 cases of viper bite –with special reference to the administration of antivenin-. J Jpn Soc Clin Surg. 1988; 49: 1923-1928.

47. Decision in Tottori district court on July 12, 1990. Hanrei Jihou 1990, 1367, 87-98.

48. Ameno S, Ameno K, Fuke C, Ijiri I, Tsunenari S. Detection of anti-horse serum antibodies produced in snake bite patients by injecting antivenin. Jpn J Acute Med. 1989; 13: 219-224.

49. Okamoto O, Oishi M, Hatano Y, Kai Y, Goto M, Kato A, et al. Severity factors of Mamushi (Agkistrodon blomhoffii) bite. J Dermatol. 2009; 36: 277-283.

50. Morine N, Oshiro A, Izumi Y, Kudaka J. Study of Habu antivenom based on human immunoglobulin: An overview. Ann Rep Okinawa Pref Instit Health Environ. 2016; 50: 57-61.

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