Management of Snakebite and Research
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Report and Working Papers of a Seminar
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The Seminar on Management of Snakebite and Research was jointly sponsored by the WHO Regional Office for South-East Asia and the Department of Medical Research (Lower Myanmar) on 11-12 December 2001 at the Department of Medical Research, Yangon.

In this seminar, various aspects of research on Russell’s viper bite, namely epidemiology, prophylaxis, first-aid, immunodiagnosis, clinical management, antivenom storage and research on Russell’s viper venom, antivenom and toxoid were presented and discussed.

The efforts of the organising committee, participants, and WHO temporary advisers towards the successful conduct of the seminar and preparation of the proceedings are gratefully acknowledged.
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Part 1
Report of the Seminar
The seminar, convened at the Department of Medical Research (DMR) was attended by physicians from tertiary referral hospitals, divisional and township hospitals throughout the snakebite endemic areas of Myanmar, DMR staff, Dr. S Kumari (SEARO), Prof. RDG Theakston (Liverpool School of Tropical Medicine) and Prof. DA Warrell (University of Oxford) (WHO Temporary Advisors).

The aims of the seminar were to review current knowledge of the epidemiology, clinical features, diagnosis, treatment and prevention of snakebites in Myanmar.

Epidemiology

In Myanmar, snakebites may kill 1 000 people each year and are a major cause of acute renal failure. Some survivors are left with permanent physical handicap, chronic renal failure and chronic hypopituitarism. It is predominantly a disease of occupation – rice farmers and plantation workers are bitten on the feet and less commonly, on the hands. Seasonal variations in incidence are related to agricultural activity (peaks in the ploughing and harvesting seasons). Species responsible for frequent severe bites are Russell’s viper, cobras and kraits. Rare severe bites are by King cobras and sea snakes. Frequent, usually mild, bites are by pit vipers (Trimeresurus and Ovophis species).

Clinical Features

Only about 50% of people bitten by snakes are envenomed. The “dry bites” result from mechanically ineffective or perhaps “defensive” bites.

Local envenoming (swelling, bruising, blistering, necrosis) is most severe in bites by vipers and cobras, and is minimal in krait bites.

Systemic envenoming includes shock, bleeding, incoagulable blood, acute renal failure (Russell’s viper); paralysis (krait); paralysis with local envenoming (cobra); muscle breakdown and paralysis with renal failure (sea snake).

A unique effect of Russell’s viper bite in Myanmar is haemorrhagic infarction of the anterior pituitary (Sheehan’s like syndrome) resulting in acute or chronic pituitary failure.

Case fatality and clinical features of Russell’s viper varies in different regions of Myanmar.

First Aid Treatment

Most traditional first aid methods (incisions, tattooing, tourniquets, black “snake stones”, electric shocks, suction, herbal remedies) are ineffective and even harmful. The most
Effective methods are immobilization of the bitten limb and transport to hospital on a stretcher. For Russell’s viper bites, pressure pad with immobilization has proved safe and capable of preventing systemic uptake of venom.

For neurotoxic elapid bites (Krait, Cobra, King cobra) pressure - immobilization using a long crepe bandage is recommended.

Hospital Treatment

Resuscitation, diagnosis of the biting species (by examining the dead snake or by inference from clinical signs and simple bed-side tests) are followed by the decision whether or not to give antivenom, ancillary treatment and rehabilitation.

Myanmar Pharmaceutical Factory formerly produced a freeze dried mono-specific antivenom which neutralized 2 mg of Russell’s viper venom per ml. Recently, a lower titre liquid antivenom has been issued, neutralizing 0.6 mg per ml venom.

Clinical trials have not yet been carried out to determine the initial dose of new antivenom. The old MPI antivenom was effective at an initial dose of 40 ml (80 ml for severe cases).

Indications for antivenom treatment are under review. In Myanmar, much antivenom is wasted by being given to non-envenomed patients.

The old MPI antivenom was commonly pyrogenic and there were some anaphylactic reactions. Reactogenicity of the new MPF antivenom is unknown. Treatment of acute renal failure in Russell’s viper victims can save many lives. Peritoneal dialysis is available in Yangon, Mandalay, Magway and in a few township hospitals. Haemodialysis is an option only in tertiary referral centres such as Yangon General Hospital.

Assisted ventilation is needed in victims of neuotoxic envenoming. Basic skills of endotracheal intubation are essential in township doctors.

Diagnosis of Biting Snake

Hospital staff must be trained to recognize the medically- important species of snakes, in case the dead snake is brought with the patient.

Rapid tests include the 20 minutes whole blood clotting test, a new dot blot colloidal dye dipstick, EIA test (developed at DMR) and urine tests including NAG and simple protein assessment by dipstick.

New Strategies to Combat Snakebite in Myanmar

The occupational risk of Russell’s viper bite can be reduced if farmers and other agricultural workers can be persuaded to wear snake-proof boots and gloves. Effective protective boots and gloves have been manufactured by DMR and tested in a pilot study. Pressure pad with immobilization is a very promising first aid method for Russell’s viper bite.
Early use of antivenom by health workers, administered by intramuscular or intravenous injection is an important idea that should be tested for safety and feasibility in properly designed randomized controlled studies.

Close communication between DMR scientists, Myanmar doctors and the national antivenom producer MPF should help to ensure that the potency and safety of MPI’s traditionally excellent antivenom is maintained.

To compensate for the known variations in venom antigenicity and composition in different areas of Myanmar, venom used to immunize horses in antivenom production should be a pool of samples from snakes of different lengths (and hence ages) and from different parts of the country.

The advantages of freeze-drying (lyophilization) antivenom (prolonged shelf-life) must be balanced against the advantage of liquid antivenom (cost and ease of administration). In rural areas, liquid antivenom can be kept cool in the water pot room.

Because of problems with demand and supply, Myanmar is now faced with the prospect of importing antivenom (e.g. from India). However, the foreign antivenoms show low potency in laboratory mouse tests compared to the old standard MPI lyophilized Russell’s Viper Antivenom and there are no data on clinical efficacy or safety (reactogenicity).

The basis for all these new initiatives is education of doctors, nurses and members of the community.

The recommendation from the meeting were very much in line with those of the WHO Meeting on Antivenom held in NIBSC, Potter’s Bar, London 7-9 Feb, 2001, reported by the temporary advisors.

**Conclusions and Recommendations**

1. Population based studies are needed in Myanmar to establish the true incidence, mortality and long-term morbidity of snakebite.
2. More research is needed on the distribution of venomous snake species in Myanmar perhaps in collaboration with the Department of Forestry.
3. Improved first aid methods, notably the pressure-pad/immobilization method should be implemented.
4. Early deployment of antivenom in health stations by specially trained health assistants should be explored.
5. Snake diagnosis using the new DMR dot blot technique should be implemented.
6. Initial dose, efficacy and reactogenicity of the new liquid MPF antivenom should be studied as a matter of urgency.
7. Use of the 20 minute whole blood clotting test (20 WBCT) to diagnose and control the dose of antivenom should be promoted.
8. Education of doctors, nurses, health assistants, other medical personnel and members of the community should be promoted as a priority.
Management of Snakebite and Research

**Recommendations by the participants**

- Population based study of snakebites – incidence, morbidity & mortality
- Definition of a pilot area
- Training of personnel (Midwives, Lady Health Visitors)

**Snake survey**

- Venomous snake survey (in collaboration with Ministry of Forestry, Agriculture)

**First aid**

- Health education
  - community awareness and health education (Township Medical Officer, Township Health Officer)
  - (Dos & don’ts first aid)
- To confirm effectiveness of local compression and immobilization
- IM injection – anterior part of thigh

**Improved management of systemic complications**

**Antivenom**

- Early antivenom
- Improve quality of antivenom
- Increase production – horses, training facilities
- Clinical trial of Myanmar Pharmaceutical Factory antivenom
- Monitor side effects

**Peritoneal dialysis**

- Capability building for peritoneal dialysis in township level
- Comparative study – peritoneal dialysis and haemodialysis in acute renal failure
- Effectiveness of renal dose dopamine on outcome of acute renal failure

**Management of neurotoxic envenoming patients**

- Health education First aid – intradermal antivenom?, compression
- Use of ambu bag
Raising awareness of endotracheal intubation and artificial ventilation at township hospital

**Immunodiagnosis**

- Rapid dipstick test
  - Specificity and efficacy
  - Dipstick for cobra
- N-acetyl β-D glucosaminidase in urine (NAG)
  - urinary NAG – in divisional level?
  - Correlation between urine NAG, proteinuria, urinary & serum fibrin degradation product (FDP)

**ASV Research**

- Efficacy & side effects
- Raising awareness of antivenom
- Storage – Sand pot
- Lyophilized antivenom
- Quality Control
- Increase local production
- Coordinating body for biologicals

**Antivenom guidelines**

**Venom research**

- Characterization of procoagulants
- Characterization of antigenic components from different localities (High Performance Liquid Chromatography, Mass Spectrometry)
- Raising antivenom against potent fractions

**Prophylaxis**

- Awareness of use of boots & gloves
- Subsidized rate
- To continue research work on toxoid using intradermal route
- Immunization – high risk group at high risk times
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Part 2

Working Papers
Acceptability Study of Protective Boots Among Farmers of Taungdwingyi Township

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Abstract
Acceptability of fang-proof protective boots was studied in 180 farmers of Taungdwingyi Township during the harvesting season, October 1995. The boots were provided free of charge. A preliminary survey showed that 98% of farmers knew snakebite could be prevented by working with boots on and only 72% wore them and 28% did not. At present 88% wear locally available rubber boots, which could not withstand penetration of Russell’s viper’s fangs. Following the trial, 99% enjoyed wearing the trial boots. These provide full sense of protection against snakebite. These are light, comfortable and the farmers could work with them on for the whole day either intermittently or continuously. Ninety-nine percent preferred to wear the trial boots in future and intended to use them even if these are not provided free of charge. The boots cost 350 kyats per pair. Ninety-nine percent could afford to buy them at a price of 250 kyats per pair. However, 1% preferred a lower price of 150 or 200 kyats per pair. In order to reduce the incidence of snakebite throughout the country, all-out wearing of the boots is needed and it is suggested that health education, selling and distribution of the boots directly to the users at an affordable (subsidised) price of 250 kyats or less per pair should be aimed at.

INTRODUCTION
Russell’s vipers (Daboia russelii siamensis) usually inhabit paddy fields and are plentiful between October and December, which coincides with the harvesting season (Tun-Pe et al., 1991). Most snakebites occur while the farmers are at work in the fields harvesting crops, carrying bundles of hay, while caring livestock and on return from work or after visiting friends or video houses after dark on poorly-lit roads. Majority of the bites occur between 6 a.m.– 6 p.m. and at work (Sann-Mya et al., 1998). Although antivenom is the mainstay in treatment of snakebite, there are delays of at least about 2–4 hours in getting treatment at the nearest health centre. Majority of the effective bites resulted in systemic envenoming while seeking treatment. Routinely used tourniquet was found to be ineffective in retarding spread of venom (Tun-Pe et al., 1987a). Although local compression
immobilization first-aid technique is found to be effective in delaying local spread of venom (Tun-Pe et al., 1995), still very few victims applied it.

In spite of energetic antivenom treatment, the mortality rate of snakebite remains high ranging from 5-10% in Tharrawaddy (Myint-Lwin et al., 1985) and 18% in Taungdwingyi study (Sann-Mya et al., 1998) which has an annual tally of over 300 cases. Majority of the victims are young adults (Sann-Mya et al., 1998), which are the working force of the country. Some of the victims suffered from pituitary insufficiency following recovery (Tun-Pe et al., 1987b) and needed permanent replacement therapy.

Since it is a preventable occupational hazard, it is expected that if a majority of the farmers wear protective boots at work in the field or on the road after dark, the incidence of snakebite and mortality will dramatically decrease and demand for production of expensive antivenom will be reduced. Rubber boots were introduced to farmers nearly three decades ago but because of the inconvenience, these did not receive much attention. A few have a traditional belief that footwear is prohibited in the paddy field. However, some have been using available rubber boots, which do not withstand penetration of snake fangs (personal observations) at work. It is high time that the acceptability of the new fang-proof rubber boots among farmers is studied.

**MATERIALS AND METHODS**

The rubber boots were developed through the concerted efforts of the Venom Research laboratory, Department of Medical Research, Veterinary Section of Myanmar Pharmaceutical Factory, Ministry of Industry 1 and Rubber Shoe Factory 1, Ministry of Industry 1, Yangon. Rubber Shoe Factory 1 manufactured a knee-length protective boot made of synthetic rubber compound. Several modifications in length, design of the boots and preparation of synthetic rubber compound were made in order to prepare rubber that could withstand penetration of Russell’s viper’s fangs as shown in the photograph.

Testing of the boot, that could withstand penetration of Russell’s viper’s fangs, was conducted at the Veterinary Section, Myanmar Pharmaceutical Factory. The Venom Research laboratory carried out acceptability trial of the boots among farmers.

A total of 180 pairs of boots were distributed free of charge to the participant farmers from five villages of Taungdwingyi Township (Mahti, Mahti Sanpya, Nyaungbinhla, Pandawgyi and Satthwa) with the help of local elders and health workers. Face-to-face interviews were conducted using two sets of structured questionnaires, before and after distribution of the boots to assess the acceptability of the boots in October 1995 and February 1996 respectively. The questionnaires included demographic characteristics of the users, knowledge on the use of protective boots, comfort, ability and duration of boot
wearing in a day. Affordability of the boots and future intention of continually using them were also enquired.

RESULTS

A total of 180 farmers aged between 26-61 years, from five villages of Taungdwingyi Township participated in the study. The sex ratio is 163 (91%) male: 17 (9%) female. Fifty one percent (92/180) are farm owners and 49% (88/180) daily-wage workers.

Predistribution survey on knowledge on the use of protective boots

Ninety-eight percent (177/180) knew snakebite could be prevented by working in the field with boots, however, only 72% (129/180) wore them at work in the field and 28% (51/180) did not. Among boot-users, 62% (80/129) prefer knee-length boot, 29% (38/129) ankle-length boot and 9% (11/129) assorted shoes. At present, 88% (114/129) wear locally available rubber boots and could work with them on for 5-9 hours a day in spite of discomfort in 12% (15/129). Eighty-eight percent (114/129) thought that it was convenient to wear the boots and 97% (125/129) mainly wore them during harvesting crops. The price of the local rubber boots is 300 kyats per pair. The reasons for failing to wear boots are 64.7% (33/51) could not afford to buy it, 21.6% (11/51) inconvenience at work and 13.7% (7/51) on traditional believe. Ninety-four percent (170/180) of them did not take any protective measures against snakebite in the field. Ninety-seven percent (175/180) would like to take part in acceptability boot trial if provided free of charge. In future, 96% (49/49) of non-boot users would like to wear boot namely 65% (32/49) the trial boot, 22% (11/49) knee-length rubber boots and 8% (4/49) ankle-length rubber boots.

Post-distribution survey on use of the boots

Ninety-nine percent (179/180) enjoyed wearing the trial boots provided, since these are light 6% (11/180) and comfortable and provide a sense of total protection against snakebite 91% (164/180). Ninety-eight percent (176/180) wore the boots during harvesting and walking in the field. Eighty-six percent (154/179) could work with the boots on for 5-9 hours continuously and 14% (25/179) intermittently. Nine percent of the users complained of local pain whereas 91% (159/175) did not.

The main advantages of working with the trial boots are: ability to work for a longer time than before with comfort and the assurance of complete protection against snakebite 100% (171/171).

On comparing the trial boots and in-use boots, 99% (141/143) prefer to wear the former in future and 1% the latter. They also intend to continue wearing them (99%, 179/180) after the trial even if these are not provided free of charge. Ninety-nine percent (178/180) could afford to buy them at 250 Kyats per pair and 1% (2/180) preferred a lower price of 150 or 200 Kyats per pair.
DISCUSSION

From the study it becomes clear that the boots under trial are superior to the locally available rubber boots. Although the former are slightly more expensive than the latter (350 Kyats vs 300 Kyats), a majority prefer the former because of comfort, ease in wearing and ability to work for the whole day with minimum discomfort. These provide a sense of full protection against snakebite so that the farmers could concentrate on their work and more productive work could be expected. While undergoing the trial, two participants who happened to step on Russell’s vipers at work were prevented from being bitten by the snakes.

Although there is a traditional custom of prohibiting wearing footwear in the field, a majority of farmers from Taungdwingyi have been wearing boots for decades because of scare of snakebite and its consequences. However, a proportion of farmers (28%), non-users who could not afford to buy boots are still at risk. In order to encourage universal wearing of the boots, they need to be subsidized and sold to the users at an affordable price of 250 Kyats per pair.

Since majority of the users have an intention of continually using them, they should be encouraged and the price of the boots should be kept to minimum. Since snakebite can occur even before they leave their houses (Sann-Mya et al., 1998) it is suggested that they should wear the boots before leaving the house until coming back home in the evening. No place is safe especially in snake-infested areas.

Health education targeted to the users, selling and distribution of the boots directly to the users at an affordable price will be next strategies in order to bring down snakebite morbidity and mortality rates in the country.

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We would like to thank U Thein-Pe, former factory manager and staff of Rubber Shoe Factory 1, Yangon, for their untiring efforts in preparation of snake-fang proof rubber boots, U Khin-Aung-Cho and staff of Veterinary Section of Myanmar Pharmaceutical Factory, Yangon, for conducting the biting experiments and Dr. Tin-Oo, Township Medical Officer of Taungdwingyi Hospital, the health assistants, the local health workers and the elders of the five villages of Taungdwingyi Township for their help in executing the boot trial.

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Acceptability Study of Protective Boots Among Farmers of Taungdwingyi Township
An Epidemiological Study of Snakebite and Venomous Snake Survey in Myanmar

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Abstract
A study of snakebite cases for 3 years (1998-2000) from 87 hospitals shows that the average prevalent rate of snakebite decreases from 24.6 to 17.4 x 100,000 and case fatality rate from 5.8 to 3.75%. Russell’s viper bite constitutes 60% (fatality rate 8.2%), cobra 6% (8%), green pit viper 5%, sea snake 0.4% and unknown 29% (3%). Russell’s viper bite occurs throughout the year with a definite seasonality. A majority of the bites occur during the ploughing and harvesting seasons. The age of the victims ranges from 6-76 years with high numbers of snakebite cases (49%) and case fatality (5.6%) in 15-25 years old age group. Russell’s viper bite occurs at work in the field and on the way home or to work. A majority of the bites occur in the lower limbs between 6 am-6 pm. The dead snakes brought by the victims suggest that Russell’s viper bite is most common followed by cobra bites. The study suggests that a community-based study of the epidemiology of snakebite would highlight the magnitude of the problem.

INTRODUCTION

Snakebite is endemic in six rice growing divisions of Myanmar and is an important health problem in the country. It belongs to one of the priority diseases laid down in the national health plan. It is an occupational hazard of farmers and has been one of the 11 single leading causes of morbidity and mortality during the last three decades in the country.

The epidemiological data on snakebite from 1954-88 has been reported (National Snakebite Seminar, 1989). An updated hospital-based morbidity and mortality survey on snakebite covering the period 1998-2000 will be presented. Also, an epidemiological study of snakebite cases admitted to Taungdwingyi hospital in 1994 (which has the highest incidence of snakebite in Myanmar) will be presented.

Trends in snakebite

A study of snakebite cases for 3 years (1998-2000) from 87 hospitals shows that the average prevalent rate of snakebite decreases from 24.6 to 17.4 x 100,000 and case fatality rate
from 5.8 to 3.75%. Russell’ viper (Daboia russelii siamensis) bites constitutes 60% (fatality rate 8.2%), Cobra (Naja kaouthia) 6% (8%), green pit viper (Trimeresurus spp.) 5%, sea snake 0.4% and unknown 29% (3%).

The prevalence and case fatality rate of snakebites from six divisions is tabulated in table (1). The average yearly prevalence of snakebite for three years (1998-2000) from 87 hospitals of six divisions is 24.6, 20.98 and 17.4 x 100,000 respectively. The average case fatality rate for three years is 5.8%, 4.2% and 3.75% respectively. The general trend of prevalence and case fatality rate of snakebite is decreasing.

Yearly prevalence and case fatality rate of snake bite differ between divisions (Table 1). Mandalay has high prevalent rate in 1998 and 1999 and Bago in 1999. High case fatality rate is observed in Magway (1998) and Bago in 1999 and 2000.

**SEASONAL DISTRIBUTION OF SNAKEBITE**

Snakebite occurs throughout the year with a definite seasonality. The majority of bites occur during the ploughing (May-June) and harvesting seasons (October-December). Monthly distribution of snakebite cases in two snakebite endemic divisions (Sagaing and Magwe) is shown in Figure 1.

The seasonal distribution of snakebite cases from Taundwingyi is similar to that of other endemic areas. However, with the introduction of multiple cropping, snakebite tends to occur throughout the year.

**Age and sex distribution**

Data from the epidemiological study of 181 snakebite cases admitted to Taungdwingyi hospital in 1994 are used. The average age of victims is 28 years (range 6-76 yrs). The number of snakebite cases is highest in 15-25 years age group (49%), followed by 26-45 years age group (29%). The case fatality rate is highest (5.6%) in 15-25 years, 3.6% in 6-14 years, 2.6% in 26-45 years and 1.6% in 46-76 years.

*Figure 1. Monthly distribution of snakebite cases*
Time of bite

Most bites occur at work in the field while ploughing and harvesting crops and on the road, on the way home or to work. Seventy-five per cent of the bites occur during the day (6 a.m.-6 p.m) and 25% after dark (6 p.m.-6 a.m). The number of bites occurring during forenoon and afternoon of day and in late evening is approximately the same. Most farmers start their work before dawn and work the whole day. That may explain the high incidence of bites during the day because of the long hours of exposure to risk. The work involved is: reaping grass, harvesting crops, paddying and minding livestock. Ninety-three per cent of late evening bites occurred on the way home from work after dark, attending livestock in the sheds in insufficient light, guarding harvested crops in the field and visiting friends. Seven per cent of the bites occurred early in the morning among those who have to tend paddy fields when tide comes in at night, for example, night watchman.

Site of bite

Eighty-eight percent of the bites occur in the lower limbs and 12% in the upper limbs. Bites occur in those engaged in plugging corn, reaping grass, harvesting crops, sesame and groundnut, chopping grass, and also in those engaged in activities like: ploughing, minding and feeding livestock, night watchman, cutting trees for firewood.

Biting habit

Russell’s vipers in the paddy fields or in the bundled crops or hay are responsible for most bites during the harvesting season. Bites also occur during ploughing when snakes are dug up. Cobra bite is few compared to Russell’s viper bite and occurs among firewood fetchers. Chinese krait bite usually occurs at night while persons are sleeping on the ground. Sea snakebite occurs among fish sorters.

Venomous snake survey

Species of snakes

A total of 375 dead snakes brought by the snakebite victims from 28 hospitals (including those sent by basic health staffs) of 7 divisions (Yangon, Bago, Mandalay, Magwe, Sagaing, Ayerawaddy and Tanintharyi) and 2 states (Kachin and Shan) were studied. Ten were non-venomous and 365 were venomous. Of the venomous species, 322 were Russell’s viper (Daboia russelii siamensis), 28 Cobra (Naja kaouthia), four B. multicinctus, one B. candidus, one B. fasciatus, four Tr. erythrurus, two Tr. purpureomaculatus, two Tr. monticola and one Tr. stegneri. Among the non-venomous were two Elaphidae radiata, two Ptyas mucosus, one Chrysopelia ornata and five Homolopsis buccata.

Snakebite occurs in all geographical regions but Russell’s viper bite is endemic in rice growing areas namely Mandalay, Sagaing, Yangon, Bago, Magwe and Ayerawaddy divisions. Cobra bite occurs in Shan State, Mandalay, Bago, Magwe, Yangon and Rakhine divisions. Although green pit vipers are found elsewhere, only 4 species were collected namely
Trimeresurus erythrurus from Yangon, Tr. purpureomaculatus from Bogalay, Tr. sternegeri from Moegoke and Tr. monticola from Kukkhine. Only two krait species were collected namely Bungarus candidus from Thahton, Mon states and B. multicinctus from Yangon.

DISCUSSION

The epidemiology of snakebite based on hospital returns for three years (1998-2000) shows that average yearly prevalence and case fatality rate of snakebite is decreasing but the fatality rate of Russell’s viper bite is 8.2% compared to that of endemic areas, Taungdwingyi (8.9%), Danuphyu (19.3%) and Nyaunglaybin (17.8%). (Kyaw-Than et al., 1997, Min-Than et al., 1998, Sann-Mya et al., 1998). Russell’s viper bite constitutes about 60% and a majority of unknown bites (29%) are attributed to it. The dead snakes brought by the victims also support that Russell’s viper bite is the most common followed by cobra (6%).

The high fatality rate following cobra bite (8%) in district hospitals probably reflects that the victims may have respiratory arrest on way to the hospital which could develop within 1.5 h after the bite (Viravan et al., 1986). This points to the need for awareness about the important role of life saving artificial ventilation (mouth-to-mouth or use of ambu bag). Most victims survived when they reached hospitals and were given mono-specific antivenom and artificial ventilatory support if needed or referred to the nearest health centre.

Although the green pit viper is found throughout the country, there were few reports of its bite from some States and divisions, since local people believe that it’s bite is non-lethal and do not bother to seek medical treatment which could account for the low incidence of the bite. A community-based study of the epidemiology of snakebite will reveal the true incidence of various bites.

Occasional fatal Chinese krait bite may have mistakenly been labeled as cobra bite because of neurotoxic symptoms since the snake is found in all parts of the country. Medical officers should be alerted and educated about the fact that most night bites which result in neurotoxic envenoming could be due to krait bite (Theakston et al., 1990, Tun- Pe et al., 1997) since most patients were asleep when bitten or did not see the snakes.

Table 1. Prevalence and case fatality rate of snakebites in six endemic divisions (1998-2000)*

<table>
<thead>
<tr>
<th>Year</th>
<th>Sagaing</th>
<th>Bago</th>
<th>Magway</th>
<th>Mandalay</th>
<th>Yangon</th>
<th>Ayeryarwady</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>25.94 (4.2%)</td>
<td>31.62 (4.0%)</td>
<td>37.2 (13.4%)</td>
<td>38.26 (5.0%)</td>
<td>4.32 (4.1%)</td>
<td>10.27 (4.2%)</td>
</tr>
<tr>
<td>1999</td>
<td>20.85 (4.3%)</td>
<td>33.76 (6.3%)</td>
<td>23.2 (4.8%)</td>
<td>33.3 (4.4%)</td>
<td>5.18 (1.2%)</td>
<td>9.6 (4.3%)</td>
</tr>
<tr>
<td>2000</td>
<td>19.43 (2.7%)</td>
<td>23.64 (6.4%)</td>
<td>20.2 (5.2%)</td>
<td>29.05 (3.8%)</td>
<td>5.06 (1.7%)</td>
<td>7.04 (2.7%)</td>
</tr>
</tbody>
</table>

* Case fatality rates are in the parentheses prevalence expressed in 100000.
The study highlighted that hospital-based epidemiology data may not reflect the true incidence of snakebite and not all snakebite cases seek medical treatment because of traditional beliefs and poor transportation to the health centre. It is envisaged that a community-based epidemiological study of snakebite will highlight the magnitude of the problem. Such studies have been carried out in some countries (Coombs’ et al., 1997, Hati, et al., 1991, Snow et al., 1994).

References


National Seminar on prevention and management of Russell’s viper bite, September 1989, Yangon, Burma.


The Role of Early Intravenous Antivenom in Management and Outcome of Russell’s Viper (Daboia russelii siamensis) Bite Cases

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Abstract

The role of early intravenous antivenom in management and outcome of 61 Russell’s viper (Daboia russelii siamensis) bite cases admitted to Taungdwingyi hospital from August 1996 to October 1997 was studied. The victims received the first dose of antivenom (1-4 ampoules) at rural health centers given by midwife (82%) at 1.10h ± 13 min. after the bite and remaining 0-3 ampoules totaling 4 ampoules at admission 3h ± 23min. after the first dose. A majority of the cases (75%) were given liquid antivenom. Venom antigen was not detected in 23% post-antivenom clotted cases. A venom level of 10-40ng/ml was detected at 5 hours in 47% post-antivenom clotted cases given 1-2 ampoules antivenom within 3 hours after the bite and 10-80ng/ml in 30% post-antivenom non-clotted cases within 6 hours after 1-5 ampoules of antivenom. Out of the 29 post-antivenom antigen positive clotted cases 6 developed systemic envenoming and 5 had complications. Systemic complications developed in 6/9 post-antivenom non-clotted cases (5 fatality) given 1-2 ampoules antivenom within 3 hours and second dose within 5 hours after the bite and in 4/9 of those (3 fatality) given 3-5 ampoules antivenom within 5 hours after the bite. It is highlighted that a single, early bolus dose of 4 ampoules of antivenom is more effective in preventing development of systemic complications and fatality than giving the total in two divided doses. Arrangements should be made for availability of antivenom at rural health centers and local health workers should be trained and legislated to give an adequate dose of early intravenous antivenom according to the guidelines after assessing the degree of envenoming by performing clotting test. Rapid quantitation of venom antigen level by dipstick will be helpful in the field for selection and estimation of antivenom dose.

INTRODUCTION

Russell’s viper (Daboia russelii siamensis) bite is an occupational hazard faced by farmers. Most bites occur in the paddy field while at work. Because of delay in transporting victims to the nearest health center, some patients are severely envenomed by the time they seek medical treatment. The practice of giving antivenom in the villages of Taungdwingyi,
Danuphyu and Yaekyi was reported (Tun-Pe et al., 2000a). A preliminary study on clinical significance of early antivenom on limited numbers of Russell’s viper bite patients by our group indicated that early antivenom combined with local compression immobilization first-aid technique was found to prevent development of systemic complications (Tun-Pe et al., 1998). However, administration of 4-10 ampoules of monospecific antivenom within 4 hours after Russell’s viper bite failed to prevent development of renal failure had been reported (Myint-Lwin et al., 1985). This study evaluated role of pre-hospital antivenom in management and outcome of Russell’s viper bite patients.

**MATERIALS AND METHODS**

Since early administration of antivenom in snakebite cases plays an important role in management, some antivenoms are provided at rural health centers by the health authority and midwives are permitted to give the first dose of intravenous antivenom to the victims and refer them to the hospital. We took the opportunity to study the role of early intravenous antivenom in management and outcome of Russell’s viper bite cases. Farmers also stock antivenom at room temperature in the villages for emergency use. Local health workers were recruited into the study and the guidelines for antivenom therapy, management of its untoward effects and how to perform clotting test were explained. The degree of envenoming was assessed by the extent of local swelling and clotting status. They were instructed to do clotting test before giving antivenom. One midwife was provided with four ampoules of lyophilized antivenom and one 20 ml disposable syringe. Antivenom could be provided to 15 local health workers only. According to the guidelines, 4 ampoules should be given to systemic envenomed cases. They were instructed to fill up the clotting test results in the forms and return the used antivenom vials for exchange with new antivenom at the hospital along with the patients. Snakebite cases admitted to Taungdwingyi hospital from August 1996-October 1997 were studied.

Routinely, at admission, a total dose of 4 ampoules of antivenom was given to all snakebite cases. A 20-minute whole blood clotting test (Warrell et al., 1977) was performed on admission post-antivenom samples in order to assess the clotting status. Serum was saved onto filter paper strips, air dried, sealed in plastic bags and transported to the Venom Research Laboratory, Department of Medical Research, Yangon for determination of venom antigen level by Enzyme immunoassay technique (Tun-Pe et al., 1991). Clinical details of the patients were recorded in standard proforma.

**RESULTS**

A total of 61 snakebite cases given 1-5 ampoules of antivenom in the villages were available for study. A majority (74%) of the victims were 31-years old males who were bitten while at work (54%) and walking home or in the field (46%). The victims received 1-5 ampoules of antivenom at 1.10h ± 13min (10minutes-4hrs 30minutes) after the bites and the remaining 0-3 ampoules (totaling 4 ampoules) at admission 3h ±23min. (45minutes-10hrs 20minutes excluding one in 25 hours) after the first dose. Two systemic envenomed cases treated with 4 ampoules antivenom received an additional 4 ampoules of it in hospital.
Clinical details of the cases

Clinical details, antivenom timing and development of systemic complications of the cases are presented in Table 1

Clotting test and antivenom therapy at the villages

In 19 out of 61 cases (31%) clotting test was carried out on the victims in the field before giving antivenom (11 clotted and 8 non-clot cases). Of 11 clotted cases, nine received 2 ampoules and the remaining 3 ampoules. Of eight systemic envenomed cases, one ampoule was given to one, two ampoules to 3, four ampoules to 3 and five ampoules to one.

Forty-six cases received liquid (75%), 14 lyophilized (23%) and one mixed (liquid and lyophilized)(2%) antivenoms. The antivenom was administered intravenously at the rural health center (60)(98%) and at home (1)(2%) by mid-wife (50)(82%), health assistant (10)(16%) and general practioner (1)(2%).

Thirty nine percent (24) received the first dose of antivenom within 30 minutes following the bite, 57% (35) in one hour, 87%(53) in 2 hours, 93%(57) in 3 hours, 98%(60) in 4 hours and 100% (61) in 4 1/2 hours.

Venom neutralization following liquid and lyophilized antivenom

Since venom antigen could be detected in 4 clotted and 4 systemic envenomed cases following 1-4 ampoules of lyophilized antivenom, no attempt was made to compare its neutralizing efficacy with that of liquid antivenom.

<table>
<thead>
<tr>
<th>Post ASV clotting status</th>
<th>No. of Subjects</th>
<th>ASV 1st dose (amp)</th>
<th>ASV 1st dose (h)</th>
<th>ASV 2nd dose (h)</th>
<th>Venom level after 1st dose ASV (ng/ml)</th>
<th>Systemic envenomating (n)</th>
<th>Systemic complications</th>
<th>Fatality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clotted</td>
<td>25</td>
<td>1-2</td>
<td>0.52 (0.10-2.30)</td>
<td>2.12 (0.045-4.45)</td>
<td>17.4 (10-40)</td>
<td>S-1</td>
<td>SA-2</td>
<td>SAB-2</td>
</tr>
<tr>
<td>Clotted</td>
<td>4</td>
<td>3-4</td>
<td>1.21 (0.15-3.30)</td>
<td>2.55 (0.105-4.00)</td>
<td>12.5 (10-20)</td>
<td>1</td>
<td>SA-1</td>
<td>0</td>
</tr>
<tr>
<td>Non-clot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-clot</td>
<td>9</td>
<td>1-2</td>
<td>1.27 (0.030-3.00)</td>
<td>4.06 (2.30-4.40)</td>
<td>51.2 (10-60)</td>
<td>9</td>
<td>S-2</td>
<td>SAR-2</td>
</tr>
<tr>
<td>Non-clot</td>
<td>9</td>
<td>3-5</td>
<td>3.27 (0.030-4.30)</td>
<td>6.14 (210-1020)</td>
<td>48.8 (40-70)</td>
<td>9</td>
<td>S-2</td>
<td>SA-1</td>
</tr>
</tbody>
</table>

* No venom antigen was detected in 14 admission post-antivenom cases.
ASV=antivenom, S=shock, SA=shock+albuminuria, SAB=shock+albuminuria+haemorrhagic anifestations, SAR=shock+albuminuria+renal failure
Post-ASV venom antigen levels

Post-antivenom clotted cases

In 43 post-antivenom clotted cases treated with 1-4 ampoules of antivenom, venom antigen was not detected in 14 and was detected in 29. A venom level of 10-40ng/ml was detectable up to 5 hours in cases given 1-2 ampoules of antivenom within 3 hours after the bite and 10-20ng/ml in those given 3-4 ampoules of antivenom within 4 hours (Table 1).

Six of 29 venom antigen positive post-antivenom clotted cases developed systemic envenoming (5 with complications) during the course of treatment (Table 1). Shock developed in 5 with or without albuminuria and haemorrhagic manifestations such as haematemesis, malena, haematuria and shock on day two. All recovered from shock.

Post-antivenom non-clot cases

Eighteen post-antivenom cases had nonclotting blood at admission (systemic envenoming). Venom antigen level of 10-80ng/ml was detectable up to 11 hours in all 18 post-antivenom cases presented with incoagulable blood given 1-5 ampoules of antivenom within 5 hours after the bites.

Nine received 1-2 ampoules within 3 hours and the second dose in 5 hours, of which 6 developed systemic complications such as 2 shock plus albuminuria, 2 shock plus albuminuria and renal failure, 2 shock plus albuminuria plus haemorrhagic manifestations such as gingival bleeding, haematemesis, malena, haematuria, epistaxis and subconjunctival haemorrhage. Five died of shock and renal failure.

In the remaining 9 systemic envenomed cases treated with 3-5 ampoules of antivenom within 5 hours, four developed systemic complications. Four developed shock with or without albuminuria and renal failure and 3 of them died.

Systemic complications in the latter group were milder and less in number than in the former. It is noteworthy that a patient given 3 ampoules of antivenom 30 minutes after the bite and another dose of one ampoule 2 hours 40 minutes after the first could not prevent development of systemic complications, shock, albuminuria, renal failure and death.

Clot restoration time of systemic cases treated with 4 ampoules was 16.8 hours (6-30 hours).

DISCUSSION

The study highlighted the value of early antivenom in management and prevention of development of complications of Russell’s viper bite cases. It is learnt that 39% of the victims received the antivenom within 30 minutes after the bite at rural health centers given by midwives and the 20 minute whole blood clotting test could be performed before giving antivenom in the field. Four systemic envenomed cases were given 1-2 ampoules of the antivenom instead of 4 ampoules and more than 4 ampoules of the antivenom should have been given to the systemic envenomed cases with complications where only
2 cases received an additional 4 ampoules of antivenom. It is probable that the dispensers were giving the antivenom as a first aid to the snakebite victims rather than rendering treatment to them and or because of limited antivenom stock. It is highlighted that these systemic envenomed cases should be treated early with adequate bolus dose of antivenom rather than giving the total in divided doses in order to prevent development of systemic complications.

A venom level of 10-20ng/ml detected in local envenomed cases is usually cleared 2 hours after the antivenom (Tun-Pe et al., 2000b) where 23% of the antigen negative post-antivenom clotted cases may belong to it. However, presence of venom (10-40ng/ml) in the post-antivenom clotted cases suggested that these victims probably received substantial amount of envenoming, of which 5 later developed systemic envenoming. It is suggested that a proportion of the latter could be detected early if whole blood clotting test (Warrell et al., 1977) and a rapid quantitation of venom antigen by dipstick (Aye-Aye-Myint et al., 2000) (if available) could be performed in the field in order to assess the degree of envenoming and early institution of appropriate dose of antivenom.

A majority of the cases were given liquid antivenom available at rural health centers and villages. Failure of venom clearance in the systemic cases up to 11 hours after 4 ampoules of the antivenom and poor clot restoration of the antivenom treated systemic cases suggested that the liquid antivenom used had a poor neutralizing efficacy of procoagulant activity of the venom. Use of lyophilized antivenom or proper storage of antivenom in the absence of cold chain (Aye-Aye-Myint et al., 1998) in the villages should be practiced.

Systemic complications developed in 67% of the post-antivenom non-clotted cases treated with 4 ampoules of the antivenom given in two divided doses within 4.40h carry a mortality of 56% whereas 38% of those treated with a bolus dose of 4 ampoules within 4 hours developed complications with 25% fatality. Our observation is in agreement with previous hospital based studies carried out in Tharawaddy (Myint-Lwin et al., 1985) and Taungdwingyi (Sann-Mya et al., 1998) where 38% and 44% systemic envenomed cases treated with a bolus dose of 4 ampoules of antivenom developed complications respectively. It is highlighted that early administration of a single bolus dose of 4 ampoules of intravenous antivenom to systemic envenomed cases (within 4 hours after the bite) in the field will have a better chance of preventing development of systemic complications and fatality compared to those given the total in two divided doses. Moreover, if antivenom therapy is combined with local compression immobilization technique, it will further prove to be effective in preventing development of complications (Tun-Pe et al., 1998).

In order to reduce the morbidity and mortality of snakebite, arrangements should be made for the availability of antivenom at rural health centers and local health workers should be legislated to give adequate dose of early intravenous antivenom in the field according to the guidelines, after giving them a full comprehensive training course on first aid management of snakebite including indication for antivenom therapy, management of its untoward effects, method of assessment of degree of envenoming by performing clotting test, use of quantitation of venom antigen by dip stick (if available) (Aye-Aye-Myint et al.,
2000), storage of antivenom in the absence of cold chain in villages (Aye-Aye-Myint et al., 1998) and promotion of wearing protective boots and gloves (Tun-Pe et al., 1998) by the farmers.

ACKNOWLEDGEMENTS

We would like to thank the local health workers from Taungdwingyi township health authority and Dr. Tin-Oo, Township Medical Officer of Taungdwingyi hospital for their help and full support in the study.

References


Trial of Efficacy of Local Compression Immobilization First-Aid Technique in Russell’s Viper (Daboia russelii siamensis) Bite Patients

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Abstract
The traditional first aid techniques used for snakebite victims are ineffective and result in some complications. The search for an effective safe and first aid technique is essential. Local compression immobilization technique was found to be effective in delaying spread of “mock’s venom, NaI131” and radiolabel Russell’s viper (Daboia russelii siamensis) venom in human volunteers and monkeys respectively. The extended study of its efficacy in 15 prospective Russell’s viper bite cases demonstrated that movement of the venom was retarded while the pad was in place (up to 1 hour) and a rise of the venom level of 10-40 ng/ml was observed following its release. Further elucidation of its efficacy was tested in the field on 20 Russell’s viper bite cases. The pad was applied on average 1.12 hours (5 min to 7 hours) after the bite for 3.40 hours (30 min to 9 hours). Venom levels measured at the hospital before and at 15 and 30 minutes after release of the pad (n10) showed a rise of 5 to 30 ng/ml of the venom following its release. Movement of venom antigen was retarded in all cases (n9) whose venom levels were measured at 15 and 30 minutes with the pad in place. Pad or immobilization alone is not effective in delaying spread of the venom. The incidence of local necrosis (8%) following use of the pad was comparable to that of the systemic cases without the pad. No ill effects were observed following application of the pad for as long as 9 hours. Local blackening seen in 10% cases was most likely the result of a local venom effect.

INTRODUCTION
Russell’s viper (Daboia russelii siamensis) bite is an occupational hazard of our farmers and carries a mortality rate of about 10%. Most snakebite patients take from 2 hours to days to get to the nearest health center. Delay in getting antivenom combined with use of ineffective first aid (Tun-Pe et al., 1987) lead to systemic envenoming by the time they seek medical
treatment. Local compression immobilization technique was found to be effective in delaying spread of “mock’s venom, NaI$^{131}$” and radiolabel Russell’s viper (Daboia russelli siamensis) venom in human volunteers and monkeys respectively (Tun-Pe et al., 1994). In this communication, the efficacy and ill effects of the local compression immobilization technique in retarding spread of the venom in prospective Russell’s viper bite cases admitted to Tharawaddy hospital (Tun-Pe et al., 1995) and field trial of the technique in Russell’s viper bite victims (Tun-Pe et al., 2000) will be presented.

MATERIALS AND METHODS

**Trial of local compression immobilization technique in prospective Russell’s viper bite cases.**

A prospective study of the efficacy of applying local pressure by compression pads in retarding the spread of venom was carried out on suspected Russell’s viper (Daboia russelli siamensis) bite cases admitted to Tharawaddy hospital during November and December 1989, 1990 and 1993 (Tun-Pe et al., 1995) and was approved by the Institutional Ethical Review Committee of the Department of Medical Research, Ministry of Health, Myanmar. For ethical reasons, we studied suspected Russell’s viper bite cases with clottable blood who would normally have been kept under observation for 24 hours after the bite. The possible side effects of the pad treatment and the merits of immobilization of the limb in delaying spread of venom were explained to the patients and informed consent was obtained. The trial was stopped if the patient could no longer tolerate the pad or developed incoagulable blood during the trial.

For the trial the patient was supine on a bed and a rubber pad (Fig. 1) with a piece of cotton bandage was applied over the site of bite with the bandage hand tight. For bites on toes and finger, a smaller rubber pad with a piece of cotton bandage was used. The affected limb was immobilised with a splint. The experiment lasted 1 hour, but in one patient the trial was extended to 2 hours to study its efficacy in retarding venom movement and the side effects following prolonged application. Serum samples were collected onto filter paper strips, air dried and later transported to the Venom Research Laboratory at the Department of Medical Research for determination of venom antigen level by enzyme immunoassay technique (Tun-Pe et al., 1991).

**Field trial of the local compression immobilization technique in Russell’s viper bite victims**

A field trial of efficacy of the local compression immobilization technique and its ill effects was carried out in 40
villages of Taungdwingyi township with the help of local health workers who were demonstrated the use of the first aid rubber pad (Fig. 1) and immobilization technique. Its merits were explained to them with the view that they will impart the knowledge to local farmers (Tun-Pe et al., 2000). A total of 800 pads, 20 pads with instruction per village, were distributed to the farmers through the health workers. The potential victim was advised to apply it soon after the bite, immobilize the bitten limb with a splint and to go to the nearest health station.

The study was approved by the Institutional Ethical Committee of the Department of Medical Research, Yangon, Myanmar. Clinical features, including degree of tightness of the pad, immobilization of the limb and progress of the patients were recorded in a standard proforma. A 20-minute clotting test (Warrell et al., 1977) was carried out on admission blood sample of suspected Russell’s viper bite cases. Routinely, the medical officer at the hospital releases the tourniquets first and gives antivenom intravenously. Following the release of tourniquet(s), two blood samples of 15 minute apart were taken after release of the pad while waiting to give antivenom. Since a retrospective analysis of the serum samples of the patients suggested a rise in venom levels following release of the pad, for ethical reasons, two samples, 15 minutes apart, with the pad in place were collected from the remaining patients. The pad was released after antivenom therapy. The sample collection and determination of venom antigen level by enzyme immunoassay technique were the same as in the previous study (Tun-Pe et al., 1991).

RESULTS

*Trial of the local compression immobilization technique in prospective Russell’s viper bite cases*

A prospective study of the efficacy of the local compression immobilization technique in retarding spread of venom was carried out on 23 Russell’s viper bite cases. Fifteen of the 23 cases had an increase of 10-40ng/ml in serum venom antigen level following release of the pad (Figure 2); the central movement of venom antigen was retarded in 13 of them. In the remaining 7 locally envenomed antigenaemic cases (venom level 10-20ng/ml), the venom antigen disappeared from the circulation while they were undergoing the pad trial (data not shown). (Tun-Pe et al, 1995).
Field trial of local compression immobilization technique in Russell’s viper bite cases

Venom antigen was detected in the serum of 19 out of 26 pad-applied cases. The age of the patients ranged from 10-60 years. Tourniquets were applied in 76% and immobilization in 13 (3 properly done). It took 1 hour ± 12 minutes (5 minutes-7 hours) to apply the pad and was applied for 3 hours 40 minutes ± 15 minutes (1/2 – 7 hours). The patients traveled 16 ± 2.7 km (0.8-45 km) to get to nearest health station.

The local features of the pad-treated cases were pain (67%), tenderness (58%), swelling (56%), blackening (10%), bleeding (8%), necrosis (8%) and bruising (6%). Eighty four percent (16/19) of the antigen-positive cases developed systemic envenoming. Spontaneous systemic bleeding occurred in 19% of systemic cases, hypotension (25%), oliguria (31%), peri orbital edema (13%), renal failure (13%), malena (6%), epistaxis (6%), hematuria (6%) and fatality (9.5%).

A rise of 5 to 30 ng/ml of venom level was measured before and after release of the pad (n10), and central movement of venom antigen was retarded in all cases (n9) whose venom levels were measured 15 minutes apart with the pad in place (Figure 3).

Mean admission venom levels of local and systemic cases were 27 ng/ml (range 10-40 ng/ml) and 64 ng/ml (50-80 ng/ml) respectively. Since proper immobilization has carried only in three cases, no attempt was made to compare the initial venom levels of immobilized and non-immobilized groups. Pyrogenic reactions following the antivenom therapy were observed in 28% of the cases.

DISCUSSION

The principle of local compression immobilization first aid technique was adapted from the Monash method (Anker et al., 1982). The effectiveness of the technique in retarding spread of mock venom and radiolabel Russell’s viper venom from the site of injection was demonstrated in human volunteers and monkeys (Tun-Pe et al., 1994). Its efficacy in retarding central movement of the venom from the site of the bite is further supported by the results obtained from the studies carried out on prospective Russell’s viper bite cases (Tun-Pe et al., 1995) and field trial of the technique on Russell’s viper bite cases (Tun-Pe et al., 2000).
Since the mean admission venom level of the non-immobilized systemic cases (64 ng/ml) was comparable to that of the systemic cases (61 ng/ml) of the Russell’s viper bite cases admitted to the Taungdwingyi Hospital (Sann-Mya et al., 1998), it is suggested that the pad alone is not effective in delaying spread of the venom (Sutherland et al., 1979). Since a majority of cases applied the pad on an average 1 hour following the bite, it is expected that a certain amount of injected venom must have already been absorbed into the circulation depending on the activity of the bitten limb. Delay in the pad application and lack of immobilization lead to rapid absorption of the venom, resulting in systemic envenoming with high admission venom levels. It has been suggested that even walking after upper limb envenoming will inevitably lead to systemic envenoming despite first aid measures (Howarth et al., 1994).

It is very likely that local concentration of the venom in the pad-applied cases could lead to local necrosis. However, its incidence (8%) is comparable to that observed in systemic envenomed Russell’s viper bite cases (10%) studied in Taungdwingyi (Sann-Mya et al., 1998). Local blackening (10%) was due to the local venom effect since it was also observed in cases without the pad. Local pain was twice more common in the pad-treated cases because of local concentration of the venom. However, the intensity of pain was not severe, amounting to a demand for removal of the pad. No ill effects were observed following prolonged, up to 6-9 hours application.

An effective first-aid technique is essential since victims have to travel 1.6 km (average) to get to the hospital. It was observed that one ampoule (10 ml) of intravenous antivenom given to the Russell’s viper bite patients combined with local compression immobilization first aid applied at villages prevented development of systemic complications (Tun-Pe et al., 1998). Because of insufficient number of pads distributed to the farmers, its application was delayed for one hour in some cases. However, a cotton pad of a similar thickness made from strips of cotton from personal wear (longyi) could be used in place of the pad on site (Fig 1). In spite of health education, the majority of snakebite victims still fail to immobilize their limbs.

References
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Intramuscular Antivenom Administration as an Effective First-Aid Measure in Management of Snakebites

Win-Aung
Diagnostics and Vaccine Research Centre, Department of Medical Research (Lower Myanmar), Yangon

Abstract
Effectiveness of intramuscular (IM) anti-snake venom (ASV) administration immediately after the bite at the site of the incident as a first aid measure in the field followed by standard hospital management versus standard hospital management alone in the therapy of Russell’s viper (Daboia russelii siamensis) bite patients was studied in two township hospitals. It was found that there was a definite reduction in the number of patients with systemic envenomation i.e. disseminated intravascular coagulation (DIC), clinical proteinuria, oliguric acute renal failure (ARF), systemic bleeding, hypotension and fatality rate of Russell’s viper bite victims who had received an initial IM ASV prior to the hospitalization, compared to those who had not. These studies showed that IM ASV could be administered as an effective first aid measure at the site of the incident in place where no facility for giving IV ASV therapy prior to hospitalization. Nevertheless, it should be noted that IM injection is to be used only as a first aid measure and is not a substitute for administration of intravenous ASV, an ideal route of anti-venom therapy. Therefore, it could be suggested that in the management of Russell’s viper bite victims in Myanmar, administration of IM ASV immediately after the bite may be of value as a first aid measure in the field where IV route of ASV is impossible and/or transport to hospital is likely to be delayed for more than 2 hours.

Russell’s viper (Daboia russelii siamensis) bite envenomation has been a serious medical problem in Myanmar for many decades. Although post-bite administration of monospecific anti-venom against Russell’s viper (RV) venom, a product of Myanmar Pharmaceutical Factory (MPF), is the effective and widely used specific treatment of the victims, the morbidity and mortality rates result from RV bite envenomation are considerably high in our country. One of the reasons may be delayed antivenom therapy probably due to the late arrival at the hospital since almost all the snakebite cases occur in paddy fields which are far away
from hospital and where an immediate administration of intravenous (IV) anti-snake venom (ASV), i.e. standard route of administration is impossible. It was reported in previous clinical studies that late arrival to hospital increases mortality. Therefore, an alternative measure to IV ASV therapy is the intramuscular (IM) injection of ASV, to be given immediately after the bite at the site of incident prior to hospitalization as a first aid measure in situations where IV administration is impossible and/or transportation to hospital is likely to be delayed. During the last decade, many attempt have been conducted by the DMR Scientific Group on Snake Bite research on the effectiveness of IM administration of ASV in the management of RV bite victims in Myanmar.

During the early half of the last decade, preliminary studies on IM administration of ASV on experimentally envenomed animals were extensively carried out and reported by DMR, showing that immediate administration of anti-sera by IM route was effective enough to reduce lethality of envenomed experimental mice.\textsuperscript{1,2} However, the amount of ASV required for achieving an equal potency was found to be 4-fold over the IV dosage\textsuperscript{3}.

Prior to the clinical trial, efficacy of IM injection of ASV was tried on 15 human subjects by giving 10 ml antivenom (i.e. one-fourth of IV dosage) and antibody levels in their circulation at various time intervals after injection were determined. It was found that certain amount of circulating antibodies to neutralize 100 mg of venom (i.e. an average venom level found in the circulation of RV bite cases) was achieved one hour after IM administration. Some amounts of antibodies were also attained half an hour after IM injection\textsuperscript{4}.

In 1992, a clinical trial of the efficacy of IM ASV administration immediately after bite as a first aid measure in the field followed by standard hospital management versus standard hospital management alone in the management of Russell’s viper bite patients was carried out in Tharyarwady Township, Bago Division, with the active participation of Basic Health Staff (BHS) who served as the mobile task force. Prior to the snakebite season, they were trained in the technique of IM anti-sera administration. Each BHS was given a pair of ASV ampoules, disposable syringes with needles, and methylated spirit swabs. They were stationed at the respective village tracts near the site of the farming activities, and were instructed to give a total of 10 ml ASV (5 ml to each buttock) intramuscularly to a victim within 2 hours after a bite in the field. Those who had received IM ASV in the field and then been transferred immediately to the Township hospital for further standard hospital management were regarded as test cases. Those who had not come to BHS, had not received IM ASV and been directly hospitalized after a bite were regarded as control cases. Patients of both the test (n=82) and control (n=34) groups were then given the same hospital management. The complications following RV envenomation and clinical outcomes of these victims were also noted and the results were summarized as below\textsuperscript{5}.

It is apparent that there was a definite reduction in the number of patients with systemic envenomation i.e. disseminated intravascular coagulation (DIC), clinical proteinuria, oliguric acute renal failure (ARF), systemic bleeding, hypotension and fatality rate of Russell’s viper bite victims who had received an initial IM ASV prior to hospitalization compared to those who had not. Besides, blood venom antigen levels at the time of admission were found to be significantly less in in test cases compared to controls,
presumably indicating that a certain amount of circulating venom introduced by the snakebite had already been neutralized by the ASV given intramuscularly prior to hospitalization. This study, therefore, showed that IM ASV could be administered as an effective first aid measure at the site of incident where no facility was available for giving IV ASV therapy prior to hospitalization.

In 1996, this promising study was therefore extended as a research-cum-action programme in Meiktila township, Mandalay Division, where snakebite morbidity and mortality rates are also very high. A total of 12 test cases and 82 control cases were involved in this study. The results are shown below.

<table>
<thead>
<tr>
<th>Clinical and biochemical features</th>
<th>Control (n=82)</th>
<th>Test (n=34)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIC (Incoagulable blood) (no)</td>
<td>34 (41.5%)</td>
<td>7 (20.5%)</td>
<td>p&lt;0.03</td>
</tr>
<tr>
<td>Clinical proteinuria (no)</td>
<td>33 (40.2%)</td>
<td>6 (17.6%)</td>
<td>p&lt;0.02</td>
</tr>
<tr>
<td>Oliguric (ARF (no)</td>
<td>18 (21.9%)</td>
<td>1 (2.9%)</td>
<td>p&lt;0.03</td>
</tr>
<tr>
<td>Systemic bleeding (no)</td>
<td>11 (13.4%)</td>
<td>1 (2.9%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypotension (no)</td>
<td>6 (7.3%)</td>
<td>Nil</td>
<td>NS</td>
</tr>
<tr>
<td>Change from coagulable to incoagulable state in hospital (no)</td>
<td>13 (15.9%)</td>
<td>Nil</td>
<td>p&lt;0.02</td>
</tr>
<tr>
<td>IV ASV administration required (no) in hospital</td>
<td>36 (43.9%)</td>
<td>8 (23.5%)</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Cases requiring peritoneal dialysis (no)</td>
<td>12 (14.6%)</td>
<td>1 (2.9%)</td>
<td>NS</td>
</tr>
<tr>
<td>Death (no)</td>
<td>7 (8.5%)</td>
<td>1 (2.9%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>8.1±9.6</td>
<td>5.7±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Blood venom antigen level on admission (ng/ml)</td>
<td>46.3±21.7</td>
<td>20±13.3</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Maximum serum creatinine level (mg/dl)</td>
<td>3.1±2.8</td>
<td>1.4±1.0</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Proteinuria &gt; 10 g/L (no)</td>
<td>18 (21.9%)</td>
<td>2 (5.9%)</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

In 1996, this promising study was therefore extended as a research-cum-action programme in Meiktila township, Mandalay Division, where snakebite morbidity and mortality rates are also very high. A total of 12 test cases and 82 control cases were involved in this study. The results are shown below.

Although most of the findings were not statistically significant, marked clinical significance was observed in the tests compared to the controls. The findings of this study also confirmed the effectiveness of IM ASV as a first aid measure in the field in the management of RV bite patients.

The IM administration of anti-sera to snakebite patients has not been routinely recommended by WHO. However, in Myanmar, almost all the snakebites occur in paddy fields which are far away from health centres and transport to hospital is likely to be delayed for more than 2-4 hours. Besides, the Basic Health Staff who encounter the snakebite patients soon after the bite have no permission to give IV administration. Therefore, IM ASV should be administered to the Russell’s viper bite patients at the site of
incident as a first aid method in places where there is no facility for giving IV ASV therapy prior to hospitalization. Nevertheless, it should be noted that IM injection is to be used only as a first aid measure and is not a substitute for administration of IV ASV. Therefore, those victims who have been injected with IM ASV must be transferred to the hospital where facilities for further management are available.

From the above studies, it could be recommended that in the management of Russell’s viper bite victims in our country, administration of IM ASV immediately after the bite may be of value as a first aid measure in the field where IV route of ASV is impossible and/or transport to hospital is delayed for more than 2 hours. It is therefore expected that the results of these studies will be of great help to the basic health staff of various districts and townships who are currently engaged in the management of RV bite victims. Hence, these findings could be considered in formulating national guidelines for the management of Russell’s viper bite patients in Myanmar.

References

Intramuscular Antivenom Administration as an Effective First-Aid Measure in Management of Snakebites


Clinical Features of Russell’s Viper (Daboia russelii siamensis) Bite Cases Admitted to Six Township Hospitals of Snakebite Endemic Divisions of Myanmar

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2Department of Medicine, Institute of Medicine (1), Yangon
Senior Medical Officers from 3Taungdwingyi, 4Danuphyu, 5Kyauske, 6Yaekyi, 7Myinmu, and 8Nyaunglaybin hospitals, Department of Health

Abstract

Clinical features, antivenom response and outcome of Russell’s viper (Daboia russelii siamensis) bite cases admitted to six township hospitals namely Danuphyu, Yaekyi (Ayerawaddy division), Nyaunglaybin (Bago), Myinmu (Saging), Kyauske (Mandalay) and Taungdwingyi (Magwe) in one calendar year were studied. Majority of the victims are young males bitten on the lower limb while at work in fields. It takes 3.44hrs (5 minutes-46 hrs) to get to the hospitals and have to travel 6.45 miles (0.25-41 miles). Forty-four percent brought dead snakes for identification. Eighteen percent received pre hospital antivenom. The length of the snake responsible for the bites ranges from 10-110cm. Twenty-eight percent of the bites are not envenomed, 29% local and 43% systemic envenoming. Forty-two percent of the systemic cases developed complications. Venom antigen levels of local envenoming cases range from 10-50ng/ml and that of systemic 20-100ng/ml. In general, common local and systemic features of Russell’s viper bites are observed in all cases, however, bruising is more common in (Yaekyi, Danuphyu and Taungdwingyi), necrosis in (Taungdwingyi, Kyauske and Danuphyu), blackening in (Danuphyu and Myinmu), gum bleeding, epistaxis and haemoptysis in (Danuphyu), haematemesis, malena and albuminuria (Danupgyu and Yaekyi), malena (Kyauske and Taungdwingyi), hypotension and conjunctival oedema (Nyaunglaybin), conjunctival haemorrhage (Kyauske and Danuphyu), renal failure (Danupgyu and Nyaunglaybin) and high mortality (12.5-19.3%) in (Kyauske, Danuphyu and Nyaunglaybin). Traditional first aids are still practised in all cases except Taungdwingyi and Myinmu. In 54% of the antivenom treated systemic cases, clot restoration time takes 12hrs-48hrs. Pyrogen reaction is observed in 50% of antivenom treated cases. Variation in clinical features of the cases, variable antivenom response and clot restoration time highlighted that venom used for raising antivenom for common use should be widely pooled.
INTRODUCTION

Snakebite is an occupational hazard of our farmers. People in six of 14 states and divisions are engaged in rice farming. Bites occur during the ploughing and harvesting seasons; however, with the introduction of multiple cropping, contacts between snakes and man is increased. Mono-specific antivenom manufactured by Myanmar Pharmaceutical Factory is recommended for treating Russell’s viper bite cases throughout the country. Common clinical features include: systemic bleeding, hypotension and renal failure. Russell’s viper bite has been studied in Tharawaddy, Taungdwingyi, Magwe, Myinmu, Danuphyu, Nyaunghlanbin and Kyauksae (Aung-Myint et al., 1998, Kyaw-Than et al., 1997, Min-Than et al., 1998, Myint-Lwin et al., 1985, Myint-Soe et al., 1997, Sann-Mya et al., 1998, Saw-Naing, 1983). Geographical variation of biological properties of Russell’s viper venom (Aye-Aye-Myint et al., 1993, 1994, 1995, Sann-Mya et al., 1994, Tun-Pe et al., 2000) and variable performance of mono specific Russell’s viper antivenom in neutralizing venoms from different localities (Tun-Pe et al., 1999) have been reported. The objective of the study is to determine the possibility of geographical variation of clinical features, antivenom response and outcome of Russell’s viper bite cases admitted to six township hospitals from snakebite endemic divisions.

MATERIALS AND METHODS

The study was conducted on snakebite cases admitted to six township hospitals, from five snakebite endemic divisions namely, Danuphyu and Yaekyi (Ayerawaddy), Nyaunghlanbin (Bago), Myinmu (Sagaing), Kyauksae (Mandalay) and Taungdwingyi (Magwe) (Figure) in one calendar year (between 1995-98).

Clinical details of the cases were recorded in standard proforma. Twenty-minute whole blood clotting test (Warrell et al., 1977) was used to assess the degree of envenoming. Guidelines for indication of antivenom were explained to medical officers before the trial. Clotting test was done at 2h intervals for 24h in cases presented with clottable blood on admission and for those presented with incoagulable blood, it was repeated at 6h intervals after antivenom till normal clot restoration was restored. Serum was collected on to filter paper strips and venom antigen levels were measured by enzyme immunoassay technique at the Venom Research Laboratory of the Department of Medical Research (Tun-Pe et al., 1991).
RESULTS

A total of 294 Russell’s viper bite cases admitted to six township hospitals were studied. The average age of the victims is 27 yrs (7-75yrs) with male to female ratio of 227:67. Twice as many victims are bitten during daytime compared to night bites. Most are bitten on lower limbs and interval between bite and admission is 3.44hrs (5minutes-46hrs). Average distance travelled is 6.45 miles (0.25-41 miles). Forty-four percent of the cases brought dead snakes for identification. Eighteen percent received pre hospital antivenom. It takes 2.14hrs (20minutes-1230hrs) to get pre hospital antivenom in the villages. Snakes responsible for the bites measured from 10-110cm. Clinical details of the patients are presented in Table 1.

Table 1. Clinical details of 294 Russell’s viper bite cases

<table>
<thead>
<tr>
<th></th>
<th>Upper Myanmar</th>
<th>Lower Myanmar</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>43</td>
<td>24</td>
<td>294</td>
</tr>
<tr>
<td>Sex M:F</td>
<td>33:10</td>
<td>20:4</td>
<td>227:67</td>
</tr>
<tr>
<td>Bite day: night</td>
<td>29:14</td>
<td>11:13</td>
<td>196:98</td>
</tr>
<tr>
<td>Age</td>
<td>27 (7-71)</td>
<td>24 (13-60)</td>
<td>27 (7-75)</td>
</tr>
<tr>
<td>Work: walk</td>
<td>37:6</td>
<td>16:8</td>
<td>206:88</td>
</tr>
<tr>
<td>Site lower: upper limb</td>
<td>26:17</td>
<td>20:4</td>
<td>243:51</td>
</tr>
<tr>
<td>Time Bite/adm: (h)</td>
<td>1.50 (025-815)</td>
<td>2:40 (020-2130)</td>
<td>3.44 (005-4600)</td>
</tr>
<tr>
<td>Dist traveled (mile)</td>
<td>8 (0.25)</td>
<td>8 (1.8-23)</td>
<td>6.45 (0.25-41)</td>
</tr>
<tr>
<td>Snake brought</td>
<td>39/43</td>
<td>-</td>
<td>119/270 (44%)</td>
</tr>
<tr>
<td>Pre hospital ASV%</td>
<td>-</td>
<td>-</td>
<td>44/245 (18%)</td>
</tr>
<tr>
<td>Pre hospital ASV time (h)</td>
<td>-</td>
<td>-</td>
<td>2.14 (0.20-1230)</td>
</tr>
<tr>
<td>Snake’s length (cm)</td>
<td>29 (23-50)</td>
<td>62 (26-102)</td>
<td>30 (10-50)</td>
</tr>
</tbody>
</table>

Local features and symptoms of the cases are shown in Table 2. Local features like bruising are more common in Yaekyi (23%), Danuphyu (23%) and Taungdwingyi (27%), necrosis in Kyauksae (13%), Taungdwingyi (10%) and Danuphyu (9%), blackening in Danuphyu (45%) and Myinmu (14%).

Degree of envenoming of the cases is shown in Table 3. Twenty-eight percent of the cases are not envenomed, 29% local and 43% systemic envenoming. Forty-two percent of the systemic cases developed complications. Mean initial venom antigen level of local
envenomed cases ranges from 20-26ng/ml (total range 10-50ng/ml) and systemic 61-71ng/ml (total range 20-100ng/ml.) In 54% (37-67%) of the systemic envenomed cases, clot restoration time takes 12hrs-48hrs. Pyrogen reactions are observed in 50%(21-82%) of the antivenom treated cases.

**Table 2. Local features and symptoms of Russell’s viper bite cases**

<table>
<thead>
<tr>
<th>Features and symptoms</th>
<th>Upper Myanmar</th>
<th>Lower Myanmar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myinmu n=43</td>
<td>Kyauksae n=24</td>
</tr>
<tr>
<td>Bruising %</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Necrosis %</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td>Blackening %</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Epigastric pain %</td>
<td>-</td>
<td>55</td>
</tr>
<tr>
<td>Vomiting %</td>
<td>32</td>
<td>55</td>
</tr>
</tbody>
</table>

**Table 3. Degree of envenoming of the Russell’s viper bite cases***

<table>
<thead>
<tr>
<th>Venom level (ng/ml)</th>
<th>Upper Myanmar</th>
<th>Lower Myanmar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myinmu n=43</td>
<td>Kyauksae n=24</td>
</tr>
<tr>
<td>Degree of envenoming</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non (%)</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Local (%)</td>
<td>72</td>
<td>21</td>
</tr>
<tr>
<td>Systemic (%)</td>
<td>19</td>
<td>62</td>
</tr>
<tr>
<td>Systemic + Compli. (%)**</td>
<td>1/8</td>
<td>47</td>
</tr>
<tr>
<td>Local enven. Mean (range)</td>
<td>23 (10-45)</td>
<td>24 (10-40)</td>
</tr>
<tr>
<td>Systemic enven. Mean (range)</td>
<td>64 (50-70)</td>
<td>71 (55-100)</td>
</tr>
<tr>
<td>Clot restoration Time &gt;6h %</td>
<td>67 (12-14h)</td>
<td>57 (12-48h)</td>
</tr>
<tr>
<td>Pyrogen reaction %</td>
<td>21 (9/43)</td>
<td>67 (16/24)</td>
</tr>
</tbody>
</table>

* number of cases  
** Calculation based on systemic cases  
enven.=envenoming  
Compli.=complications

Development of systemic complications following the bites is shown in Table 4. In general, systemic bleeding and complications are observed in all cases; however, some features are more common in some localities such as gum bleeding, epistaxis, haemoptysis
Management of Snakebite and Research

Table 4. **Systemic bleeding and complications of Russell’s viper bite cases**

<table>
<thead>
<tr>
<th>Features</th>
<th>Myinmu n=43</th>
<th>Kyauksae n=24</th>
<th>Taungdwingyi n=134</th>
<th>Danuphyu n=31</th>
<th>Yaekyi n=34</th>
<th>Nyaunglaybin n=28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gums %</td>
<td>*1</td>
<td>-</td>
<td>-</td>
<td>28</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Haematemesis %</td>
<td>*1</td>
<td>-</td>
<td>7</td>
<td>22</td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>Malena %</td>
<td>-</td>
<td>27</td>
<td>18</td>
<td>6</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Epistaxis %</td>
<td>*1</td>
<td>-</td>
<td>-</td>
<td>17</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Haemoptysis %</td>
<td>*1</td>
<td>-</td>
<td>-</td>
<td>44</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Haematuria %</td>
<td>-</td>
<td>20</td>
<td>13</td>
<td>39</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>Conj. Hage. %</td>
<td>*1</td>
<td>13</td>
<td>2</td>
<td>11</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Hypotension %</td>
<td>-</td>
<td>27</td>
<td>22</td>
<td>28</td>
<td>19</td>
<td>47</td>
</tr>
<tr>
<td>Albuminuria %</td>
<td>-</td>
<td>3</td>
<td>10</td>
<td>56</td>
<td>77</td>
<td>4</td>
</tr>
<tr>
<td>Conj. Oedema %</td>
<td>-</td>
<td>13</td>
<td>18</td>
<td>11</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>Renal failure %</td>
<td>-</td>
<td>16.6</td>
<td>11</td>
<td>22.5</td>
<td>11.7</td>
<td>21.4</td>
</tr>
<tr>
<td>Fatality %</td>
<td>-</td>
<td>12.5</td>
<td>8.9</td>
<td>19.3</td>
<td>8.8</td>
<td>17.8</td>
</tr>
</tbody>
</table>

* number of cases

Table 5. **First aid method used in Russell’s viper bite cases**

<table>
<thead>
<tr>
<th>First Aid Method</th>
<th>Upper Myanmar</th>
<th>Lower Myanmar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myinmu</td>
<td>Kyauksae</td>
</tr>
<tr>
<td>Tourniquet %</td>
<td>90</td>
<td>92</td>
</tr>
<tr>
<td>Herb %</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>Suction %</td>
<td>-</td>
<td>58</td>
</tr>
<tr>
<td>Incision %</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>Tattoo %</td>
<td>-</td>
<td>58</td>
</tr>
<tr>
<td>Chicken Pag %</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Immobilization %</td>
<td>70</td>
<td>33</td>
</tr>
</tbody>
</table>

(Danuphyu), haematemesis, haematuria and albuminuria (Danuphyu and Yaekyi), malena (Kyauksae and Taungd-wingyi), conjunctival haemorrhage (Kyauksae and Danuphyu), hypotension and conjunctival oedema (Nyaunglaybin), renal failure (Danuphyu and Nyaunglaybin). Fatality rate is high in Kyauksae, Danuphyu and Nyaunglaybin (12.5 –19.3 %).

Use of first-aid in snakebite is shown in Table 5. Traditional first aid, tourniquets are used in all cases, however application of herbal medicine, suction, incision and tattooing are widely used in all except Taungdwingyi and Myinmu. Chicken pad is used in Taungdwingyi only.

**DISCUSSION**

The results of the studies support observations made in earlier studies (Aung-Myint et al., 1998, Kyaw-Than et al., 1997, Min-Than et al., 1998, Myint-Lwin et al., 1985, Myint-Soe
et al., 1997, Sann-Mya et al., 1998, Saw-Naing, 1983). Majority of the victims are young males, bitten on lower limb while at work in the field. Eighteen percent of the cases received pre hospital antivenom, suggesting that farmers are aware of the importance of receiving early antivenom following the bite. Some victims developed systemic envenoming after receiving 1-2 ampoules of pre hospital antivenom. Fifty percent of nonenvenomed cases were given 1-4 amp. antivenom. Twenty nine percent of the cases were locally envenomed and were given 1-4.5amp. antivenom. It has been reported that 1-2 amp. antivenom could clear a venom level of 10-50ng/ml in 2h (Tun-Pe et al.,2001). Systemic and systemic cases with complications were given 1-12 amp.of antivenom (30 amp. in one) in single or divided doses. A single bolus dose is preferred which will help in rapid neutralization of incoming venom. It is high time the antivenom policy of treating Russell’s viper bite cases is reviewed.

In vitro observation of geographical variation of Russell’s viper venom (Aye-Aye-Myint et al., 1993, 1994, 1995, Sann-Mya et al., 1994, Tun-Pe et al., 2000) supports the present observation of variation in clinical features of Russell’s viper bite cases from different localities. Variation in clinical features of Russell’s viper bite cases from two close localities could be attributed to quantitative difference in venom properties. Observation of variation in biological properties of the Russell’s viper venom from two close localities, Taungdwingyi and Aunglan, (Sann-Mya et al., 1994), Danuphyu and Yaekyi (Aye-Aye-Myint et al., 1993) and Tharawaddy and Letpadan (Aye-Aye-Myint et al., 1995) has been reported. Albuminuria and haematuria are commonly observed in Danuphyu/Yaekyi, perhaps their venoms possess potent nephrotoxic factors which needs further elucidation.

Degree of envenoming depends on effectiveness of the snake in injecting venom into the victim, size of the snake and intervals between bite and admission or antivenom therapy. Seventy-six percent of the bites in Yaekyi developed systemic envenoming (Table 3), suggesting that young snakes of 53cm in length (20-66cm) are very efficient in injecting venom into the victims. However, 72% of the bites in Myinmu resulted in local envenoming indicating that young snakes of 29cm in length (23-50cm) with short fangs are less effective in injecting venom into the victims.

The low mortality rate in Myinmu could be attributed to: the snakes responsible for the bites being young ones (23-50cm), routine administration of 4 amp. of antivenom combined with practice of immobilization (70%) of the affected limb. On the other hand, the high mortality rate (19%) in Danuphyu could be due to high proportion of cases (67%) developed systemic envenoming with complications following the bites. High incidence of pyrogen reactions (50%) and poor clot restoration property (54%) of the antivenom could be remedied by use of pyrogen-free potent antivenom preparation. A widely pooled venom rather than locally available venom should be used for raising antivenom for common use (Tun-Pe et al., 2000). The traditional first aid techniques that should be abandoned are still widely practised in Kyauksae and lower Myanmar. Use of chicken pad as a first-aid should be discouraged. Local compression immobilization first aid technique should be promoted for which effective health education is needed.

References


Effect of the Snake’s Length and Recent Feeding on Venom Antigenaemia and Severity of Envenoming in Russell’s Viper (Daboia russellii siamensis) Bites

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4 Centre for Tropical Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, UK

Abstract

An enzyme immunoassay technique was used for detection of venom antigen levels in the diagnosis of 311 suspected Russell’s viper bite cases in Myanmar, 181 of whom (58%) had systemic envenoming. Russell’s viper venom was detected in the sera of 175 (56.3%). Among 175 of these patients, who failed to bring the dead snake, EIA achieved a specific diagnosis of Russell’s viper envenoming in 101 (58%). Stomach contents were examined in 101 Russell’s vipers responsible for bites. The presence of prey, usually a rodent, in the snake’s stomach, indicating that it had eaten recently, did not influence the severity of envenoming, the initial venom level, or the percentage circumference increase and the extent of local swelling in the bitten limb. One hundred and fifty-five Russell’s vipers responsible for bites showed a bimodal distribution of total lengths. The smaller snakes had probably been born that year. Longer snakes were responsible for more severe envenoming, a shorter interval between the bite and the detection of incoagulable blood, and more extensive local swelling with a greater percentage circumference increase of the bitten limb; but their bites were not associated with higher initial venom antigenaemia or a greater risk of developing acute renal failure.

INTRODUCTION

Snakebite is a major medical problem in Myanmar, resulting in more than a thousand deaths per year (Myint-Lwin et al., 1985). Especially in the rice-growing area, the majority of bites are attributed to Russell’s viper (Daboia russellii siamensis), the venom of which causes local swelling, haemostatic disturbances, shock and acute renal failure (Myint-Lwin et al., 1985). A similar clinical syndrome can result from envenoming by green pit vipers (Trimeresurus spp.) and by the Malayan pit viper (Calloselasma rhodostoma), which may
Management of Snakebite and Research

occur in the south of the country. When a snakebite victim fails to identify or bring to hospital the snake responsible, the choice of antivenom will depend on the clinical history and symptoms, which may not be specific. An enzyme immunoassay (EIA) was used to measure Russell’s viper venom antigen in serum for retrospective assessment of the clinical diagnosis and exploration of the pathophysiology and treatment of envenoming. The notion that smaller snakes and those that have eaten recently might be less venomous has been tested in a large number of cases.

MATERIALS AND METHODS

We studied 413 patients with proved or suspected bites by Russell’s vipers admitted to Tharrawaddy township hospital, 80 km north of Yangon, Myanmar, during 5 rice-harvesting seasons (November-January) in 1984-1988; 155 patients (38%) brought the dead snake. The total lengths of the dead snakes were measured. The stomach was opened through a ventral incision to see if the snake had fed recently. In the patients, severity of envenoming was graded clinically as none, local or systemic according to criteria established in a previous study (Myint-Lwin et al., 1985). The 20-minute whole blood clotting test, a simple bedside test, was used to monitor coagulopathy (Warrell et al., 1977). Quantitation of venom antigen in serum was done by EIA technique already described (Tun-Pe et al, 1991a).

RESULTS

Detection of venom antigen by Enzyme Immunoassay

Out of 311 snakebite victims studied by EIA, Russell’s viper venom was detected in the sera of 175 (56.3%). Among those 311 studied, 136 brought the dead snakes and the remaining 175 had failed to bring the dead snake. Out of 175, EIA achieved a species diagnosis of Russell’s viper bite in 101 (57.7%).

Lengths of snakes responsible for bites and correlation with severity of envenoming

The distribution of the total lengths (tip of snout to tip of tail) of 155 snakes responsible for bites was bimodal, indicating the presence of 2 distinct populations (< 375 mm and > 550 mm long)(Fig.1).

Bites by 112 snakes < 375 mm long resulted in 21 cases of systemic and 91 cases of non-systemic envenoming. Bites by 43 snakes > 550 mm long caused 26 cases of systemic envenoming (Fig. 1). Systemic envenoming was significantly correlated with bites by longer snakes ($\chi^2 = 27.60, p<0.005$). However, the mean initial venom levels in 9 patients with systemic envenoming resulting from bites by snakes < 375 mm long were similar to those in 15 patients bitten by snakes > 550 mm long (68.66± 20.51 [1 standard deviation] and 59.53 ± 23.04 ng/ml respectively) ($t= 0.98, 0.20<P<0.40$). The intervals between admission to hospital and development of incoagulable blood in 9 patients bitten by snakes < 375 mm long and 16 bitten by snakes > 550 mm long were significantly different (means 6.20 and 2.15 h respectively) ($t= 3.91, P<0.001$).
To assess the relationship between the length of snakes and the severity of local envenoming, 101 patients bitten by snakes varying in length from 125 to 1100 mm were studied. Lengths of snakes correlated with the extent of local swelling ($r = 0.3$, $0.001 < P < 0.005$) and with the percentage circumference increase of bitten limbs ($r = 0.2$, $0.025 < P < 0.05$) (Spearman's rank correlation).

Out of total 413 patients we studied, 119 patients were systemic envenomed and of them, 59 (50%) developed acute renal failure. Among the 44 patients with systemic envenoming who brought the dead snake, 27 developed renal failure and 17 did not. The lengths of snakes whose bites were responsible for causing renal failure ranged from 223 to 1120 mm, there was no significant correlation between snake length and development of renal failure (Mann-Whitney U test, $z = 1.90$, $P = 0.0574$).

### Effects of recent feeding (prey found in the snake’s stomach) on severity of envenoming

In 101 of the snakes responsible for bites the stomach was opened to discover if the snake had recently eaten (Fig. 2).

Sixty-seven of the snakes had empty stomachs. The stomachs of 34 snakes contained recently ingested prey: rodents (29), frogs (3), geckoes (1) or birds (1). The snakes having eaten recently had no effect on the development of local swelling in the patients whom they bit (Mann-Whitney U test, $z = 0.3194$, $P = 0.37$); on the degree of local envenoming reflected by the percentage circumference increase of bitten limbs compared with the control limb ($t = 0.56$, $0.4 < P < 0.5$); or, in the patients who developed systemic envenoming, on the patients’ mean initial serum venom concentrations ($61.90 \pm 26.11$ ng/ml) ($t = 0.69$, $0.4 < P < 0.5$).
Twelve out of 34 patients bitten by snakes whose stomachs contained prey and 22 out of 67 bitten by snakes with empty stomachs developed systemic envenoming. Bites by 67 snakes, 45 with empty stomachs and 22 whose stomachs contained prey, caused no envenoming (Fig. 2). Using the continuity corrected Yates’s $\chi^2$ test, no correlation was found between the presence or absence of prey in the snakes’ stomach and the degree of envenoming ($P>0.9$).

**DISCUSSION**

EIA was used for retrospective immuno-diagnosis in snakebite victims and estimation of venom levels in the victims. EIA achieved diagnosis in 58% of patients with suspected Russell’s viper bites who failed to bring the dead snake. Two populations of Russell’s vipers were found to be responsible for bites during the rice harvest (November-January); one measuring 125-375 mm, and the other 500-1125 mm (Fig. 1). The mean length of 30 four-week-old Russell’s vipers reared at the Myanmar Pharmaceutical Industry snake farm was $218 \pm 12.5$ mm (1 standard deviation). In India and Sri Lanka, the length of newborn D. russelii has been reported as 216-279 mm (Wall, 1907). Therefore, it seems likely that the population of smaller snakes responsible for bites in Tharrawaddy area had been born that year. The length of the snake was associated with the time taken for evolution of incoagulable blood in patients who developed systemic envenoming. However, bites by small (young) vipers also led to systemic envenoming (Fig. 1). Young vipers have less venom reserve than adult specimens, but their venom has greater lethal defibrinogenating and oedema-inducing activity (Tun-Pe et al., 1995), which may explain the development of systemic envenoming and renal failure following effective bites by these small specimens.
Age-related variations in procoagulant activity have been observed with *Bothrops moojeni* (Furtado and Kamiguti, 1985), *Crotalus horridus* (Bonilla et al., 1973) and *C. atrox* (Reid and Theakston, 1978).

There is a correlation between venom yield and length of Russell’s vipers. About half the content of the venom gland can be injected in defensive bites by large specimens (Tun-Pe and Khin-Aung-Cho, 1986), and we predict that most effective bites by large Russell’s vipers would result in rapid defibrinogenation. The extent of local swelling and percentage circumference increase of the bitten limb were found to correlate with the length of the snake in a previous study (Myint-Lwin et al., 1985). This correlation was confirmed by the present study.

There is a widespread belief that snakes are less harmful after they have eaten and that bites sustained in the morning, after the snake’s nocturnal hunting and feeding, will be less deadly than those in the evening or night. Our observations indicate that the extent of local swelling, percentage circumference increase of the bitten limb, degree of envenoming, and admission venom level of systemic cases were not influenced by whether the snake had eaten recently. We have carried out other studies (Tun-Pe et al 1991b) which support the view that the amount of venom (40-230 mg) left in the glands of a large Russell’s viper after four successive hunting bites would still be sufficient to cause systemic envenoming if the snake then bit a human.

**References**


Clinical Management of Russell’s Viper Bites

Tin-Nu-Swe

Department of Medical Research (Lower Myanmar)

Abstract

Antivenom is the only specific antidote to snake venom. Antivenom is indicated in all cases if any one of clinical features such as: (i) progressive local swelling at the bitten area, (ii) presence of local swelling and snake identified as Russell’s viper, (iii) spontaneous systemic bleeding, (iv) hypotension and laboratory evidences such as massive urine proteinuria, (v) incoaguable blood by whole blood clotting test is present. A single dose of 40 ml of monospecific antivenom is indicated for Russell’s viper bite patients with incoaguable blood or severe local swelling. Another dose of 40 ml is repeated if blood is still incoaguable six hrs after the initial dose. A bolus dose of 80 ml is indicated for patients with incoaguable blood, heavy proteinuria and or systemic bleeding on admission.

INTRODUCTION

Ever since the introduction of specific antiserum, antivenom therapy has been the mainstay of treatment for snakebite cases including Russell’s viper envenoming. In Myanmar the national policy is to treat all patients bitten by Russell’s viper with 40 ml of monospecific Russell’s viper antivenom (ASV) produced from Myanmar Pharmaceutical Industry (MPI)(Hla-Myint et al., 1982). This dose is based on various assumptions, and one of them is the neutralizing ability of antisera tested in mice. According to a clinical study, 40% of patients did not need antivenom, 30% (with clinical evidence of severe envenomation) required more than 40 ml antivenom to correct the coagulation defect (Myint-Lwin et al., 1985).

It has been reported that more than 75 mg of venom may be injected by adult and (less frequently) young snakes (Tun-Pe and Khin-Aung-Cho, 1986). Hence, 40 ml of the monovalent antivenom would not be adequate in most cases. Therefore, an open comparative study was conducted since a double blind trial was impracticable. The objective was to compare the efficacy of a single dose of 40 ml and 80 ml ASV in preventing serious complications. It was expected that the result obtained would provide guidelines for deciding the dose of ASV to be given.
PATIENTS AND METHODS

Proven Russell’s viper bite patients with disseminated intravascular coagulation (DIC) entered into the study. Russell’s Viper envenomation was confirmed by identification of the dead snake and/or detection of specific venom antigen in serum by EIA (Khin-Ohn-Lwin et al., 1984, Tun-Pe et al., 1991). Patients under 12 and over 60 years and pregnant and lactating mothers were not included in the study. Patients were randomly allocated to one of the following two treatment regimens:

Regimen 1

A single dose of 40 ml of ASV was given intravenously (IV) over 10 minutes. In those patients whose blood remained incoagulable six hours after the initial dose, another dose of 40 ml was given (IV over 10 minutes).

Regimen 2

A bolus dose of 80 ml of ASV (IV over 20 minutes). Lyophilized monovalent enzyme refined monospecific antivenom for Russell’s viper was used. Each individual patient was allocated to one of the above two regimens by lot.

Analysis

The two groups of patients were compared with respect to the patient’s characteristics, clinical features, progress, response and outcome. The patients under regimen one were carefully analysed to bring out the significant differences between those who needed 40 ml only and those who required additional 40 ml. Statistical analysis was done by using student “t” test (paired) and Chi square test.

RESULTS

Comparing regimens one and two

A total of 45 patients were studied. 23 of them received regimen one and 22 patients received regimen two. Characteristics and clinical features of patients were shown in Tables 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>Regimen1</th>
<th>Regimen2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>Sex ratio (male: female)</td>
<td>17:6</td>
<td>7:4</td>
</tr>
<tr>
<td>Average weight (kg)</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>Venom antigen levels (ng/ml) on admission</td>
<td>Mean (range)</td>
<td>Mean (range)</td>
</tr>
<tr>
<td></td>
<td>71 (30-110)</td>
<td>85 (50-130)</td>
</tr>
<tr>
<td>Interval between bite to antivenom (hours)</td>
<td>Mean (range)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 (1-16)</td>
<td>3 (1.5-12)</td>
</tr>
</tbody>
</table>
Clinical progress and response to treatment

Response to treatment was shown in Table 3. There was no significant difference in clinical progress between the two groups. Venom antigen was completely neutralized in both groups within six hours after the administration of ASV. More rapid clearance of venom antigen was seen in those who received a larger initial bolus dose, but the differences were not statistically significant (Table 3).

Table 3. Response to treatment with the two regimens

<table>
<thead>
<tr>
<th></th>
<th>Regimen 1 (n=23)</th>
<th>Regimen 2 (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clot restoration time (h)</td>
<td>7.6 (1.5 – 20.3)</td>
<td>7.5 (3.7 – 15.6)</td>
</tr>
<tr>
<td>Mean (Range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venom antigen clearance Time (h); mean (range)</td>
<td>1.4 (0.25 – 6)</td>
<td>1.2 (0.25 – 3.5)</td>
</tr>
<tr>
<td>No. of patients with hypotension</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>No. of patients with oliguria</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Peritoneal dialysis</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Deaths</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
The clinical progress and response to treatment in patients with systemic bleeding and heavy proteinuria (+3 and above) in the two groups are shown in Table 4. In these patients, the venom antigen clearance time was significantly shorter when a bolus dose of 80 ml was given (Table 4). But development of acute renal failure and mortality rates were similar among these two treatment groups.

Table 4. Response to treatment in patients with systemic bleeding and/or proteinuria admission.

<table>
<thead>
<tr>
<th></th>
<th>40+40 ml ASV</th>
<th>80 ml ASV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>No. of patients developing acute renal failure</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Peritoneal dialysis (no. of cases)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Deaths (no. of cases)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Venom antigen clearance time (hours) mean SEM</td>
<td>1.7 ± 1.0</td>
<td>1.1 ± 0.8</td>
</tr>
<tr>
<td>Clot restoration time (hours) mean SEM</td>
<td>10.77 7.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

P < 0.05 (Student 't' test).

Detailed analysis of regimen one (n = 23) 11/23 patients (48%) in this group received only 40 ml of ASV. None of these patients developed any major complications and none died. 12 patients had to be given additional 40 ml of ASV as their blood failed to reclot six hours after the initial dose. Close monitoring of these patients in regimen one brought out an interesting fact. Among patients requiring a second dose of ASV, eight patients (75%) had heavy proteinuria (+3 and above) and four patients (33%) had spontaneous systemic bleeding from gastrointestinal tract. This can be compared with one patient (9%) and none respectively in patients requiring no additional ASV (Tables 5 and 6).

Table 5. Characteristics of patients in regimen one on admission (comparing patients receiving 40 ml and 40 + 40 ml ASV)

<table>
<thead>
<tr>
<th></th>
<th>40 ml ASV</th>
<th>40 + 40 ml ASV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>Average weight (kg)</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
<td>Venom antigen level (ng/ ml) mean (range)</td>
<td>71 (20-110)</td>
<td>73 (50-100)</td>
</tr>
</tbody>
</table>

P < 0.05 (Student 't' test).
Hence, the following conclusions and recommendations were made as guidelines for antivenom therapy in Russell’s Viper bite patients.

**CONCLUSIONS**

1. 40 ml of monovalent antivenom of Russell’s viper was sufficient in 48% of envenomed patients without systemic bleeding or heavy proteinuria (+3 and above) on admission.

2. 33% of patients with systemic bleeding and 75% of patients with proteinuria (+3 and above) needed a bolus dose of 80 ml.

3. Patients who had received 40 ml needed another dose of 40 ml to correct the coagulation defect if they developed systemic bleeding and or heavy proteinuria or if blood failed to re-clot six hours after the initial dose.

4. There was no reduction in the incidence of acute renal failure by increasing the dose to 80 ml.

**RECOMMENDATIONS**

1. An initial bolus dose of 40 ml is recommended for envenomed patients without any systemic bleeding or heavy proteinuria on admission.

---

**Table 6. Clinical features in regime one on admission (comparing patients receiving 40 ml and 40 + 40 ml ASV).**

<table>
<thead>
<tr>
<th></th>
<th>40 ml ASV</th>
<th>40 + 40 ml ASV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local swelling number of cases</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Increase in circumference (mean SEM)</td>
<td>5.6 ± 7</td>
<td>3.6 ± 3</td>
</tr>
<tr>
<td>Regional lymphadenitis (no. of cases)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Systemic bleeding (no. of cases)</td>
<td>nil</td>
<td>4</td>
</tr>
<tr>
<td>Gum</td>
<td>nil</td>
<td>2</td>
</tr>
<tr>
<td>Haematemesis and malaena</td>
<td>nil</td>
<td>3</td>
</tr>
<tr>
<td>Total white cell count/ cumm (mean)</td>
<td>40 660</td>
<td>29 425</td>
</tr>
<tr>
<td>Platelet count/cumm (mean)</td>
<td>363 500</td>
<td>257 222</td>
</tr>
<tr>
<td>Urine protein (no. of cases)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Proteinuria &lt; plus 3</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Proteinuria plus 3&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopic harmaturia</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>RBC 50 (+1)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Rbc numerous</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(2) In patients with systemic bleeding and/or heavy proteinuria on admission, a bolus dose of 80 ml is recommended.

(3) In patients who had received the initial dose of 40 ml, another 40 ml is recommended if blood fails to reclot six hours after the initial dose, or they develop systemic bleeding or proteinuria.

References


Management of Acute Renal Failure Following Russell’s Viper Bite at the Renal Medical Unit, Yangon General Hospital

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2 Prof. and Head of Renal Medical Unit, Yangon General Hospital

Abstract

Bites by the snakes of vipersidae family are a common occurrence in Myanmar, and their clinical course is often complicated by acute renal failure. As our unit is a tertiary referral centre, most of the cases came in with established acute renal failure with advanced uraemia often with severe complications such as septicaemia, DIC and other organ failure. As a developing country with limited resources for extracorporeal renal replacement therapy (RRT) we have to rely on acute peritoneal dialysis which is an invaluable therapeutic tool. Out of 101 patients admitted in a one year period, 68 patients (67.3%) had to undergo dialytic therapy and 26 patients (25.7%) recovered with optimal conservative therapy without RRT. Seven patients signed and left because of profound severity of their illness with very slim chances of recovery. Dialytic therapy had to be provided on the fifth or sixth day of postviper bite. Mortality was 30% and, the causes of death were septicaemia 41% and myocardial failure 34%.

INTRODUCTION

Snakebite is a common occurrence in rural Myanmar and if adequately treated by specific antivenom in time and carefully monitored, the mortality could be virtually nil. Renal failure is a common event following a bite by Russell’s viper, a common snake found in Myanmar. The study aims to assess the magnitude of renal failure following Russell’s viper bite, timing for initiation of dialytic therapy, its indications and complications, and the main causes of mortality. Brief discussions about pathogenetic mechanism, renal histological changes encountered and conservative therapy are included.

MATERIALS AND METHODS

This is a retrospective study. All cases of viper snakebite admitted in the renal medical ward of Yangon General Hospital over a one year period between December 2000 and
November 2001 were included. Any patient with premorbid systemic conditions was excluded. In each case, detail clinical history was elicited and examination was performed. A close surveillance was maintained on clinical and biochemical status of the patients both before and after instituting appropriate conservative and renal replacement therapy up till full recovery.

Acute renal failure (ARF) was defined as serum creatinine more than 200 µmol/L and advanced uraemia as >800 µmol/L.

The pathogenetic mechanism in ARF following viper bite includes (1) direct venom nephrotoxicity (2) non-specific effects of envenomation, haemorrhage, hypovo-laeemia, hypotension, blood hyperviscosity, intravascular hemolysis, intravascular coagulation, rhabdomyolysis, cardiotoxicity, mediators release leading to renal ischaemia.

Renal histological changes consist of acute tubular necrosis in 70-80% of patients and bilateral cortical necrosis in 22%. Less commonly encountered lesions are acute interstitial nephritis, necrotizing vasculitis and proliferative glomerulonephritis.

MANAGEMENT OF ESTABLISHED ARF

First, reversible factors must be rapidly and exhaustively sought and treated, such as – prerenal factors: obstructive uropathy, glomerulonephritis, renal vascular and interstitial disease, intrarenal crystal precipitation.

Complications can be prevented by serial assessment of clinical and biochemical parameters.

Fluid intake – restrict to match measurable plus insensible losses in patients with normo hydration; as minimal as possible in overhydrated patients.

Electrolytes “ restrict to match measured losses: Na – (<2Gm 86 mmol / day) and K – (<1.5 Gm 40 mmol / day).

Diet -restrict protein to 0.6gm/kg/day provide nonprotein calories at least 35 kcal/kg/day, allow wt loss of 0.5lb/day, consider hyperalimentation in hyper-catabolic patients.

BIOCHEMICAL MONITORING

- Serum Na- avoid hyponatraemia by restricting free water.
- Serum K- treat hyperkalemia with NaHCO₃, glucose + insulin, kayexalate or dialysis.
- Use Ca gluconate to antagonize the cardiac effects of K⁺.
- Serum HCO₃- maintain pH at > 7.2 or HCO₃ level at 20 mmol/L or above.
- Serum PO₄ - control with Ca containing PO₄ binders.
- Serum Ca- treat only if symptomatic.
Management of Snakebite and Research


- Dialysis

The decision to start dialysis is usually made on clinical grounds and Urgent Life Saving Renal Replacement Therapy is required in: severe resistant hyperkalemia, intractable fluid overload causing pulmonary oedema, acidosis production circulatory compromise, over uraemia manifesting as encephalopathy, pericarditis, uraemic bleeding.

Dialysis is also indicated if any of the following occur: plasma K > 7 mmol/L, urea > 35 mmol/L, creatinine > 800 µmol/L, HCO₃ < 12 mmol/L, arterial pH < 7.1

RESULTS

A total of 101 patients were studied. All were in advanced renal failure (severe ARF) with or without complications. 68 patients required RRT either in the form of acute peritoneal dialysis alone or acute peritoneal dialysis followed by haemodialysis. In the remaining 33 patients, 26 patients recovered with optimal conservative therapy. Seven patients signed and left because of the irreversible nature of their complicating clinical conditions. Dialytic therapy was initiated for 42 patients (62%) on the fifth and sixth day of post viper bite and only one patient on the tenth day.

With respect to the type of dialytic therapy, acute peritoneal dialysis had to be done one time only in 55 patients, of which 14 expired, twice in 10 patients of which two expired, thrice in two patients – all recovered, and acute peritoneal dialysis followed by haemodialysis in one patient – who expired.

In the 68 patients required dialytic therapy, 43 patients recovered (63%) and 25 patients expired (37%). The main causes of death in our series were septicaemia (41%), myocardial failure (34%) followed by internal bleeding, respiratory failure and uraemia.

DISCUSSION

The incidence of ARF following viper bite is variable. It was reported to be 32% by Chugh, et al. (1984)² and only 0.99% by Zou and Zharg (1994)³ which highlight the regional differences in the distribution of types of snake. Russell’s viper is distributed widely in Myanmar and its envenomation causes defibrination, spontaneous haemorrhage, shock and renal failure. Bite – to hospital time was found an important correlate towards development of ARF and delay in seeking medical care an important factor in its development⁴. Since 38% of viper bites are blank bites, indication for antivenom therapy should be strictly followed in order to save it (Snakebite Research Group, DMR, 1999)⁵. When to initiate dialysis depends on daily catabolic load and associated complications. Hypercatabolism is usually present in the presence of frank DIC, massive tissue destruction at the bite site and steroids administration given to counteract early onset hypotension, impending myocardial failure from myocarditis and sight threatening optic neuritis. In those cases early initiation is recommended. But, prefer to do absolutely necessary only to
coincide recover of renal function with only one session of PD so that subsequent PD may not require. More frequent PD entails greater risks of complications. If renal functions do not recover after 2 or 3 sessions of PD, one must switch on to haemodialysis to contain the threat of uraemia.

Regarding indications for RRT, clinical indications such as overt uraemia manifesting as pericarditis, encephalopathy and uraemic bleeding were noted in 35 patients, pulmonary oedema in 18 patients and severe hyperkalemia in two patients. Biochemical indications due to high urea and creatinine level were seen in 13 patients. Infections and haemorrhage was seen in 10 patients undergoing peritoneal dialysis. The most common cause of mortality was sepsicaemia followed by myocardial failure. As advanced uraemia patients are mostly immunocompromised, it is not suprising that sepsicaemia accounts for the majority of death.

**CONCLUSION**

ARF is a serious condition. It depends on the severity of the underlying condition and concomitant organ failure. In the patients requiring dialysis, mortality was 40%. The most important prognostic factor is whether the kidneys are the only organ system to fail. Timely commencement of dialysis largely resolves azotaemia/uraemia and its harmful sequelae and provides adequate treatment of complicating conditions. Uraemia itself is only responsible for a minority of deaths and patients die “with” and not “because” of ARF.

**References**

Recent Advances in the Experimental, Clinical and Immunological Assessment of Antivenom

RDG Theakston
Alistair Reid Venom Research Unit, Liverpool School of Tropical Medicine, Liverpool, UK

In most cases of envenoming, the snake responsible for the accident is either not brought to the hospital with the victim or if it is, it is frequently incorrectly identified. The use of enzyme immunoassay (EIA) has proved to be very useful, in the absence of informed taxonomic identification, in establishing the identity of the snake causing the accident. Recent studies on the specificity of EIA in victims of snakebite in Ecuador have validated the use of the system which if capable of distinguishing between the venoms of even closely related Bothrops species. The main problem is the time taken to provide a diagnosis which is too long to enable the clinician to select an appropriate monospecific, as opposed to a polyspecific, antivenom.

The first stage in the assessment of an antivenom involves the use of in vivo rodent and invitro laboratory bench tests which determine whether the antivenom is effective in eliminating both general life-threatening and other pathological venom effects. Recent efforts in our laboratory have resulted in been put into replacing the discredited rodent lethality tests (LD\textsubscript{50}, ED\textsubscript{50}) which cause a great deal of pain and suffering, with a non-sentient test involving the use of immature fertile hens’ eggs.

The second, and most important, stage in testing antivenoms is the clinical assessment of efficacy as indicated by the reversal of the signs of systemic envenoming (eg. venom induced haemorrhage, coagulopathy, neurotoxicity). The amount of antivenom given to the patient is obviously related to the neutralizing efficacy of the antivenom used. Whereas in the past, treatment of patients has depended almost entirely on the individual clinician’s experience in assessing the type of snake biting the patient and the severity of envenoming, recently the development of EIA has enabled a more scientific appraisal of the situation by permitting the accurate estimation of levels of circulation-specific venom and antivenom at any time in the patient’s blood or other body fluids. It is, therefore, possible to measure the efficacy of antivenom in the neutralization and clearance of venom antigen. In Brazil, it has been shown that clinicians are treating patients with excessive amounts of highly efficient Bothrops polyspecific antivenom, with a resulting unacceptable high incidence of early anaphylactic reactions and in Sri Lanka the use of imported Indian antivenom is relatively ineffective in neutralizing the venoms of Sri Lankan snakes, demonstrating the real problem of venom variability within individual species. For example, Indian Russell’s
viper venom does not possess the neurotoxin present in the venom of the Sri Lankan species. In West Africa, the improved clearance of venom when a monospecific Echis antivenom is used in the treatment of *E. ocellatus* envenoming, compared with a polyspecific antivenom, has been demonstrated. Similar advances have been made in Myanmar.

Such clinically-based immunological studies provide an objective assessment of antivenom therapy which has not in the past been feasible.

This should result in a more efficient and controlled use of expensive antivenoms for treatment of systemic envenoming. Such studies also emphasize the importance of individual countries producing their own antivenoms for treatment of snakebite.

EIA can also be used for assessing the value of traditional and new first-aid measures such as tourniquets and the possible use of small-fragment antivenoms, administered by the intramuscular route, as a prehospital treatment for envenoming.
This was the first meeting convened by WHO on the subject of antivenoms since 1979; it was attended by 30 representatives of commercial antivenom producers, 20 involved medical and non-medical scientists, seven drug administration and quality control experts and two representatives of the European Union. The aim of the Workshop was (1) to discuss how the manufacture, efficacy and quality of antivenoms could be improved without unduly increasing the cost; (2) discuss how the availability, supply of antivenom and treatment could be advanced in the developing world (especially in sub-Saharan Africa), and (3) develop practical recommendations to achieve these aims.

The importance of snakebite and scorpion stings as public health issues was emphasized and the epidemiology, pathophysiology, clinical manifestations and treatment of snakebite was reviewed. Following a discussion of the basic immunological and pharmacological approaches to antivenom production (e.g., use of different IgG formulations, immunization techniques etc) a global review of current methods of antivenom production indicated that the most widely used method was salt precipitation and enzyme (pepsin) digestion of immune plasma to produce F(ab)2 fragment antivenoms. Other methods included the use of untreated serum, papain digestion to produce Fab fragment antivenoms and treatment of immune serum with caprylic acid to directly produce stable IgG in the absence of salt precipitation. Ion exchange and diafiltration were used by some groups to remove high molecular weight aggregates and low molecular weight contaminants as well as affinity purification to enhance purity and potency. The horse was the animal of choice for immunization, but sheep, small mammals and hens had also been used. Other matters discussed in detail included the preclinical and clinical testing of antivenoms. The former requires the development of new non-sentient systems to replace the discredited rodent lethality assays and the latter has been severely neglected over the years.

The privatization of many antivenom manufacturers which were formerly government subsidized threatens antivenom production for the developing world due to its lack of profitability. The plight of sub-Saharan Africa was particularly highlighted, but there is little
doubt that the trend would affect other regions where bites and stings are a problem. Antivenom manufactures which possessed extra capability to produce antivenoms for use in areas of need such as Africa were approached and had agreed to help to solve the immediate crisis (producers from Colombia, Costa Rica and India). Concerns about the risk of human disease from animal blood products, especially that posed by viruses or prions, was raised but there is no evidence at the moment to suggest that antivenoms may be implicated.

In the interests of improving the efficacy, safety and economy of antivenoms, it was agreed that many basic assumptions (such as the need to remove Fc fragments by enzyme digestion and to lyophilize antivenoms) should be critically re-examined and that more attention should be given to the use of clinical trials. An offer by a Brazilian manufacturer to host and to part-fund a centre to explore these issues was warmly welcomed by delegates, but it was pointed out that WHO must spearhead this initiative and approach appropriate agencies for funding.
Urinary NAG: An Indicator for Detection of Early Renal Damage in Russell’s Viper Bite Cases

Win-Aung

Abstract

Oliguric acute renal failure (ARF) is the most common severe clinical manifestation responsible for most deaths from Russell’s viper (RV) (Daboia russelii siamensis) bite in Myanmar. In these studies, the activities of urinary NAG (N-acetyl b-D glucosaminidase) excretion in 23 RV bite patients with ARF following the systemic envenomation were studied and compared with the results of other routine standard renal function tests such as urinary albumin, blood urea, serum creatinine and creatinine clearance at different time intervals after the bite. When the times of onset of the conditions, indicated by the cut-off values were compared, urinary NAG was found to be the earliest indicator of renal damage. In each type of ARF, the urinary NAG level was abnormal before changes in the values of the other indicators of renal function. It may be possible to predict the types of ARF within two hours after the bite by measuring the activities of urinary NAG. Besides, measurement of the 24-hour urinary excretion activities in the victims on the day 1 after the bite may also be useful in predicting the types of ARF. It was also noted that the significant positive correlation was observed between urinary NAG activities and circulating venom antigen levels of these victims. Besides, there was a partial correlation between urinary NAG and blood urea, serum creatinine and creatinine clearance but urinary NAG activities showed significant positive correlation with albuminuria index and proteinuria index. It is also apparent that the demonstration of the elution patterns of NAG isoenzymes in the urine of the victims, especially the presence of I-form of NAG indicated a better reflection of renal damage induced by RV envenomation. In another study, urinary NAG activities were measured in 16 proven RV bite patients confirmed by the presence of venom antigen their blood, without disseminated intravascular coagulation (DIC) (i.e. no features of systemic envenomation) and compared the results with those of other routine standard renal function tests as mentioned above. It was found that activities of urinary NAG and albuminuria index showed a considerable variation, but other standard indicators for renal function revealed no abnormality. Therefore, it is beyond doubt that these findings on urinary NAG measurement and its significance will be of great help to health personnel at various levels of health institutions for proper and improved diagnosis of envenomation and ARF in Russell’s viper bite patients in our country.
INTRODUCTION

Oliguric acute renal failure (ARF) is the commonest severe clinical manifestation responsible for most deaths from Russell’s viper (Daboia russelli siamensis) bite in Myanmar. Being a national health problem in the country, much has been studied on various aspects of laboratory diagnosis of Russell viper envenomation and its fatal complications. Recently, a great deal of interest has been focused on the utilization of the urinary enzyme, N-acetylβ-D glucosaminidase (NAG; β-N acetylglucosaminidase, EC 3.2.1.30), for early diagnosis of renal damage since the activities of urinary NAG increase before the results of other renal function tests become abnormal (Prince & Dance). Measurement of urinary NAG has been reported to be a sensitive and specific indicator of renal damage in patients with any disease reported to show renal involvement and may prove valuable for early detection of renal injury prior to manifestation by other renal function tests. This review summarizes the results of a wide range of studies on the urinary NAG activities in Russell’s viper (RV) bite patients carried out by members of the Scientific group on Snake Bite Research at the Department of Medical Research (Lower Myanmar).

1. Urinary NAG in RV bite victims with acute renal failure

The activities of urinary NAG excretion in 23 RV bite patients with ARF following the systemic envenomation were studied and compared with the results of other routine standard renal function tests. In this study, urinary NAG activities, urinary albumin, blood urea, serum creatinine and creatinine clearance were measured in these patients at different time intervals after the bite during clinical observation for 5 days. Urinary NAG was measured by using the method of Levy and Conchie (1966) in which a substrate p-nitro phenyl-N-acetyl-β-D glucosaminidase is converted by NAG present in the urine, in the presence of 0.08% of Bovine serum albumin (BSA) and 0.2 M citrate buffer, into p-nitrophenol (pNP) and N acetyl-β-D-glucosamine. After incubation for one hour at 38°C, this enzyme catalyzed reaction is terminated by the addition of an inhibitor, glycine–NaOH buffer. The liberated end product, pNP (yellow colour) was measured by spectrophotometer at 430 nm and the enzyme activity of NAG was calculated by using the molar extinction coefficient of pNP. Activities of NAG were compared with other indicators of renal function in three clinical conditions; namely non oliguric acute renal failure (ARF), oliguric ARF not requiring peritoneal dialysis (PD) and oliguric ARF requiring PD. Cut-off values to identify the three conditions were established by using the non-parametric Fisher’s exact test. Results of this study showed that urinary NAG activities were found to be raised at the time of admission at the hospital in all types of ARF.

When the times of onset of the condition, indicated by the cut-off values were compared, urinary NAG was found to be the earliest indicator of renal damage. In each type of ARF, the urinary NAG level was abnormal before changes in the values of the other indicators of renal function. Besides, it may be possible to predict the types of ARF within two hours after the bite by measuring the activities of urinary NAG as shown in the table mentioned below.
It was found that if urinary NAG activity is more than 1.1 MEU/creatinine at two hours after a bite, the outcome may be oliguric ARF. If urinary NAG activity is more than 15, the outcome may be oliguric ARF-requiring peritoneal dialysis. If urinary NAG activity of more than 28 MEU/creatinine detected at any time, the patient is definitely in the stage of oliguric ARF-requiring peritoneal dialysis (Table 1). Therefore, measurement of urinary NAG activity soon after the bite may be valuable for early diagnosis of ARF and for predicting the severity and prognosis of renal damage in patients with systemic envenomation induced by Russell’s viper bite.

### Table 1. Comparison of the times after bite when urinary NAG activities were first raised above the accepted normal maximum in different types of ARF.

<table>
<thead>
<tr>
<th>Types of ARF</th>
<th>Time (in hours after bite) of first onset of urinary NAG activities (MEU/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;1.1*</td>
</tr>
<tr>
<td>Non-olig. ARF (n=12)</td>
<td>4.45±0.88</td>
</tr>
<tr>
<td></td>
<td>(1.3-12.0)</td>
</tr>
<tr>
<td>Olig. ARF-not req. PD(n=5)</td>
<td>2.20±0.09</td>
</tr>
<tr>
<td></td>
<td>(2.0-2.3)</td>
</tr>
<tr>
<td>Olig. ARF – req PD (n=6)</td>
<td>1.98±0.43</td>
</tr>
<tr>
<td></td>
<td>(0.8-2.9)</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM; Ranges are expressed in parentheses.

*normal maximum

**cut-off point between non-oliguric acute renal failure (ARF) and oliguric ARF

***cut-off point between oliguric ARF not requiring peritoneal dialysis and oliguric ARF requiring peritoneal dialysis

2. **Urinary NAG in non-DIC cases**

It is well documented that no case has been reported with ARF and other complications occurring without disseminated intravascular coagulation (DIC) and there has been no other apparent clinical or biochemical evidence of renal functional impairment in these victims. However, since the kidney has a reserve of renal tissue, a considerable amount of structural damage can occur before functional damage becomes apparent. Therefore, it is necessary to determine whether the kidney is structurally involved in RV bite patients without DIC. In this study, urinary NAG activities were measured in 16 proven RV bite patients confirmed by the presence of venom antigen their blood, without disseminated intravascular coagulation (DIC) (i.e. no features of systemic envenomation) and compared the results with those of other routine standard renal function tests. Urinary NAG, urinary albumin, blood urea, serum creatinine and creatinine clearance were measured in these patients at different time intervals after the bite during clinical observation for five days. It was found that activities of urinary NAG and albuminuria index showed a considerable variation but other standard indicators for renal function revealed no abnormality. These findings demonstrated a direct nephrotoxic effect of Russell’s Viper Venom (RVV), although patients showed no obvious clinical manifestation of systemic envenomation or biochemical evidence of renal functional impairment. Hence, it could be concluded that structural damage to the kidney occurred in RV bite victims in the absence of DIC, confirming the urinary NAG as an early indicator of envenomation and the direct toxic effect of RVV on the kidney.
3. Significance of 24–hour urinary NAG activities

Total excretion of 24-hour urinary NAG was studied in 23 RV bite patients with acute renal failure admitted to the Tharyarwaddy township hospital. The mean ±SEM activities of 24-hour NAG excretion in patients with non oliguric ARF (n=12), oliguric ARF—not requiring PD (n=5) and oliguric ARF-requiring PD (n=6) were found to be 6.1±0.8, 8.4±1.1 and 13.2±1.4 EU/24-hour respectively at day 1 after the bite, showing significant differences. Afterwards, these activities rapidly declined and no significant differences were found to be on day 3 after the bite. The arbitrary cut-off point of urinary NAG excretion between non-oliguric ARF(n=12) and oliguric ARF (n=11) was calculated to be 8 EU/24 hours with significant p value of 0.0295. Therefore, it could be generally predicted whether RV bite patients may become non-oliguric or oliguric ARF in the later periods of the clinical course by measuring the 24-hour urinary excretion activities in the victims on day 1 after the bite.

4. Correlation between urinary NAG and circulating venom antigen levels

It is a well known fact that the severity of envenomation and of renal involvement in RV bite victims could be assessed by determination of venom antigen levels in their blood and activities of NAG in their urine respectively. The relationship between activities of urinary NAG (MEU/mg creatinine) and circulating venom antigen levels (ng/ml) in 38 known RV bite patients was determined using the correlation coefficient test. Significant positive correlation was observed between urinary NAG activities and circulating venom antigen levels of these victims with correlation coefficient (r) value of +0.53 (p< 0.05). Therefore, a simple measurement of urinary NAG activities could be utilized as an indicator of envenomation i.e an entry of venom into the systemic circulation of a victim at the hospitals where there is no facility for determination of blood venom antigen.

5. Relationship between urinary NAG and routine parameters for assessment of renal function

It is well documented in previous studies that urinary NAG is an early indicator of renal damage in RV bite victims in Myanmar. On studying the correlation of its activities with routinely measured parameters for renal function assessment in 23 RV bite victims with renal involvement, a partial correlation was found between urinary NAG and blood urea, serum creatinine and creatinine clearance with correlation coefficient (r) values of +0.31, +0.36 and −0.47 respectively. Besides, urinary NAG activities showed significant positive correlation with albuminuria index and proteinuria index with correlation coefficient (r) values of +0.91 (p<0.001) and +0.87 (p< 0.01) respectively. It is suggested that activities of urinary NAG could be utilized as one of the parameters for assessment of renal damage in RV bite patients.

6. Isoenzyme profile of urinary NAG

There are two major isoenzymic forms of NAG; A and B, and two minor intermediate forms; I1 and I2. Recently, the isoenzyme profile of NAG in urine of patients with severe renal damage resulted from RV bite envenomation was studied by using the DEAE cellulose ion-exchange chromatography and compared with those of normal controls. Although a
large amount of NAG α-form and a small amount of β-form were detected in normal urine, in pathological urine of RV bite patients with renal damage. Further increasing amount of both α and especially β-form with, in addition, a considerable amount of the intermediate I-form(s) were excreted. It is apparent that the demonstration of the elution patterns of NAG isoenzymes in the urine of the victims, especially the presence of I-form of NAG indicated a better reflection of renal damage induced by RV envenomation.

In conclusion, it is beyond doubt that these findings on urinary NAG measurement and its significance will be of great help to health personnel at various levels of health institutions for proper and improved diagnosis of envenomation and ARF in Russell’s viper bite patients in our country. Therefore, facilities for determination of urinary NAG should be encouraged in district hospitals located in snakebite endemic areas. These findings and suggestions should be considered in formulating national guidelines in management regarding the laboratory diagnosis of RV bite patients in Myanmar.

References


Geographical Variation of Biological Properties of Russell’s Viper (Daboia russelii siamensis) Venom

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²Veterinary Division, Myanmar Pharmaceutical Factory, Ministry of Industry, Yangon

Abstract

Biological properties of Russell’s viper (Daboia russelii siamensis) venoms collected from five snakebite endemic divisions: Mandalay, Magwe, (Upper Myanmar) Ayerawaddy, Yangon and Bago (Lower Myanmar) were studied by WHO recommended techniques. It is uncommon to see venom from one locality possessing all potent different biological properties. It is noted that adult Russell’s viper venom of Kyauksae is devoid of capillary permeability increasing activity (CPI) and possess poor defibrinogenating and lethal activities. Both young and adult Russell’s viper venoms of Tharawaddy possess potent CPI among the venoms studied and 92% of the colour of the young Russell’s viper venom of Danuphyu is white. Biological properties of potent young Russell’s viper venoms are comparable to that of adult. On the whole, both young and adult Russell’s viper venoms of lower Myanmar are comparatively more potent than that of upper Myanmar except in defibrinogenating and haemorrhagic activity in adult and coagulant in young. Intra and inter divisional variations in the properties of Russell’s viper venom may lead to variation in clinical features of the Russell’s viper bite victims and also performance of antivenom in these victims. Antivenom manufacturers should consider pooling venom from different localities in order to raise potent antivenom for common use.

INTRODUCTION

Russell’s viper (Daboia russelii siamensis) bite is an occupational hazard of farmers and is endemic in rice growing divisions. Geographical variation of biological properties of venom has been reported from other regions (Minton and Weinstein, 1976, Glenn and Straight, 1978, Jayanthi and Gowda, 1988, Tun-Pe et al., 1993, 2000a). Earlier studies on Russell’s viper venoms from Ayerawaddy, Magwe and Yangon divisions showed variation in the biological properties of the venoms. (Aye-Aye-Myint et al., 1993, 1994, 1995, Sann-Mya et al., 1994). Understanding of variation in biological properties and composition of venom
Management of Snakebite and Research

plays an important role in preparation of antivenom for common use. It will also help in understanding of the clinical and pathophysiology of Russell’s viper bite cases from different localities. This report concerns the study of biological properties of venoms collected from five snakebite endemic divisions of Myanmar namely Mandalay, Magwe, Ayerawaddy, Yangon and Bago.

MATERIALS AND METHODS

Russell’s vipers caught from five divisions (Figure), namely Mandalay, Magwe, Yangon, Ayerawaddy and Bago were housed separately and kept in the snake farm of Myanmar Pharmaceutical Factory. Venom milked from individual snake was lyophilized and pooled according to locality and length into young (<90 cm) and adult (>90 cm) and stored in dark at 4°C. For determination of biological activities of the venom, it was reconstituted in distilled water to a concentration of 1mg/ml, aliquoted and stored frozen at -20°C.

Biological properties of the venom such as lethality (LD₅₀), coagulant (MCD), haemorrhagic (MHD), necrotic (MND), defibrinogenating (MDD) and capillary permeability increasing activities (MCPID) were studied according to WHO recommended techniques (Theakston and Reid, 1983).

RESULTS

Biological properties of Russell’s viper venoms of Mandalay (Table 1), Magwe (Table 2), Bago (Table 3), Ayerawaddy (Table 4) and Yangon (Table 5) divisions are presented in respective tables.

<table>
<thead>
<tr>
<th>Study areas</th>
<th>Source of venom</th>
<th>Length of snake (cm)</th>
<th>Number of snakes pooled</th>
<th>LD₅₀ + f µg/mouse</th>
<th>MCD* µg/ml</th>
<th>MDD µg/mouse</th>
<th>MHD µg/rat</th>
<th>MND µg/rat</th>
<th>MCPID* µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandalay division</td>
<td>Kyauksae</td>
<td>&lt;80</td>
<td>42</td>
<td>6.45 ± 1.88</td>
<td>0.040</td>
<td>5</td>
<td>52.19</td>
<td>45.19</td>
<td>0.0660</td>
</tr>
<tr>
<td></td>
<td>&gt;80</td>
<td>40</td>
<td>14.35 ± 3.90</td>
<td>1.355</td>
<td>12</td>
<td>54.33</td>
<td>47.86</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>Magwe division</td>
<td>Thazi</td>
<td>&lt;80</td>
<td>48</td>
<td>6.30 ± 2.25</td>
<td>0.200</td>
<td>4</td>
<td>61.66</td>
<td>39.81</td>
<td>0.0955</td>
</tr>
<tr>
<td></td>
<td>&gt;80</td>
<td>40</td>
<td>3.75 ± 1.94</td>
<td>0.630</td>
<td>2</td>
<td>54.95</td>
<td>39.31</td>
<td>0.0707</td>
<td></td>
</tr>
<tr>
<td>Bago division</td>
<td>Wundwin</td>
<td>90-102</td>
<td>2</td>
<td>7.25 ± 2.51</td>
<td>3.162</td>
<td>3</td>
<td>67.61</td>
<td>56.23</td>
<td>0.01479</td>
</tr>
</tbody>
</table>

f = 95% fiducial limit
NT = not detected
Results are mean of duplicates and triplicates*
### Table 2. Biological properties of Russell’s viper venoms of Magwe division

<table>
<thead>
<tr>
<th>Source of venom</th>
<th>Length of snake (cm)</th>
<th>No. of snakes pooled</th>
<th>LD$_{50}$ f $\mu g$/mouse</th>
<th>MCD * $\mu g$/ml</th>
<th>MDD $\mu g$/mouse</th>
<th>MHD $\mu g$/rat</th>
<th>MND $\mu g$/rat</th>
<th>MCPID * $\mu g$/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taungtwingyi</td>
<td>51-89</td>
<td>9</td>
<td>7.72±2.8</td>
<td>0.426</td>
<td>3</td>
<td>41.7</td>
<td>44.7</td>
<td>0.089</td>
</tr>
<tr>
<td></td>
<td>92-106</td>
<td>4</td>
<td>3.97±2.4</td>
<td>0.501</td>
<td>1</td>
<td>30.9</td>
<td>32.4</td>
<td>0.083</td>
</tr>
<tr>
<td>Aunglan</td>
<td>78-104</td>
<td>3</td>
<td>7.9±2.4</td>
<td>1.778</td>
<td>1.5</td>
<td>23.7</td>
<td>25.1</td>
<td>0.158</td>
</tr>
<tr>
<td>Pakokku</td>
<td>84-109</td>
<td>5</td>
<td>7.06±2.3</td>
<td>0.159</td>
<td>5</td>
<td>56.2</td>
<td>52.5</td>
<td>0.141</td>
</tr>
</tbody>
</table>

$f = 95\%$ fiducial limit
Results are mean of duplicates and triplicates*

### Table 3. Biological properties of Russell’s viper venoms of Bago division

<table>
<thead>
<tr>
<th>Source of venom</th>
<th>Length of snake (cm)</th>
<th>No. of snakes pooled</th>
<th>LD$_{50}$ f $\mu g$/mouse</th>
<th>MHD $\mu g$/rat</th>
<th>MND $\mu g$/rat</th>
<th>MDD $\mu g$/mouse</th>
<th>MCD * $\mu g$/ml</th>
<th>MCPID * $\mu g$/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Letpadan</td>
<td>61-90</td>
<td>14</td>
<td>5.01±1.67</td>
<td>32.36</td>
<td>46.77</td>
<td>5.1</td>
<td>0.195</td>
<td>0.0020</td>
</tr>
<tr>
<td></td>
<td>91-120</td>
<td>10</td>
<td>3.76±1.74</td>
<td>37.15</td>
<td>55.59</td>
<td>5.1</td>
<td>0.302</td>
<td>0.0028</td>
</tr>
<tr>
<td>Thayarwaddy</td>
<td>61-90</td>
<td>14</td>
<td>4.35±1.8</td>
<td>42.17</td>
<td>44.67</td>
<td>2.5</td>
<td>1.148</td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td>91-120</td>
<td>14</td>
<td>4.67±2.0</td>
<td>42.17</td>
<td>39.81</td>
<td>2.4</td>
<td>0.251</td>
<td>0.0007</td>
</tr>
<tr>
<td>Wall</td>
<td>61-90</td>
<td>9</td>
<td>3.96±1.9</td>
<td>63.1</td>
<td>58.8</td>
<td>3.33</td>
<td>0.182</td>
<td>0.0011</td>
</tr>
<tr>
<td></td>
<td>91-110</td>
<td>13</td>
<td>5.76±2.4</td>
<td>38.46</td>
<td>31.62</td>
<td>4.2</td>
<td>0.141</td>
<td>0.0034</td>
</tr>
<tr>
<td>Daik-Oo</td>
<td>91-110</td>
<td>12</td>
<td>3.24±1.41</td>
<td>42.66</td>
<td>50.12</td>
<td>4.2</td>
<td>0.436</td>
<td>0.0080</td>
</tr>
<tr>
<td>Min Hla</td>
<td>66-81</td>
<td>10</td>
<td>18.77±7.96</td>
<td>48.98</td>
<td>36.31</td>
<td>9</td>
<td>0.072</td>
<td>0.0147</td>
</tr>
<tr>
<td>Oak Pho</td>
<td>63-90</td>
<td>11</td>
<td>4.32±3.3</td>
<td>64.57</td>
<td>45.71</td>
<td>2.5</td>
<td>0.112</td>
<td>0.0547</td>
</tr>
</tbody>
</table>

$f = 95\%$ fiducial limit
Results are mean of duplicates and triplicates*

### Table 4. Biological properties of Russell’s viper venoms of Ayerawaddy Division

<table>
<thead>
<tr>
<th>Source of venom</th>
<th>Length of snake (cm)</th>
<th>No. of snakes pooled</th>
<th>LD$_{50}$ f $\mu g$/mouse</th>
<th>MHD $\mu g$/rat</th>
<th>MND $\mu g$/rat</th>
<th>MDD $\mu g$/mouse</th>
<th>MCD * $\mu g$/ml</th>
<th>MCPID * $\mu g$/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daedaye</td>
<td>61-90</td>
<td>7</td>
<td>3.50±1.96</td>
<td>42.17</td>
<td>29.85</td>
<td>3.3</td>
<td>0.159</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>91-120</td>
<td>7</td>
<td>2.86±1.42</td>
<td>28.18</td>
<td>27.86</td>
<td>1.78</td>
<td>0.135</td>
<td>0.019</td>
</tr>
<tr>
<td>Nyaungdon</td>
<td>61-90</td>
<td>17</td>
<td>2.87±1.47</td>
<td>36.14</td>
<td>60.29</td>
<td>2.4</td>
<td>0.1</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>91-120</td>
<td>18</td>
<td>3.3±2.35</td>
<td>46.77</td>
<td>32.36</td>
<td>2.40</td>
<td>0.033</td>
<td>0.015</td>
</tr>
<tr>
<td>Danuphyu</td>
<td>71-90</td>
<td>14</td>
<td>4.42±1.19</td>
<td>30.9</td>
<td>30.9</td>
<td>4.2</td>
<td>1.995</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>91-120</td>
<td>14</td>
<td>6.38±0.98</td>
<td>30.20</td>
<td>27.54</td>
<td>5.10</td>
<td>2.291</td>
<td>0.018</td>
</tr>
<tr>
<td>Pyapon</td>
<td>91-130</td>
<td>4</td>
<td>6.44±0.54</td>
<td>23.99</td>
<td>40.27</td>
<td>2</td>
<td>0.26</td>
<td>0.020</td>
</tr>
<tr>
<td>Pantanaw</td>
<td>91-110</td>
<td>4</td>
<td>5.39±10.3</td>
<td>32.73</td>
<td>20.42</td>
<td>3</td>
<td>0.070</td>
<td>0.063</td>
</tr>
</tbody>
</table>

$f = 95\%$ fiducial limit
Results are mean of duplicates and triplicates*
It is uncommon to see venom from one locality possessing all potent biological properties. It is noted that adult Russell’s viper venom of Kyauksae is devoid of capillary permeability increasing activity (CPI) and possesses poor defibrinogenating and lethal activities.

**Venom variation among young Russell’s vipers**

Venom possessing potent biological properties from each division (Table 6), lower and upper Myanmar (Table 7) and five divisions (Table 8) are shown in respective tables.

### Table 5. Biological properties of Russell’s viper venoms of Yangon division

<table>
<thead>
<tr>
<th>Source of venom</th>
<th>Length of snake (cm)</th>
<th>No. of snakes pooled</th>
<th>LD$_{50}$ $\mu$g/mouse</th>
<th>MDD $\mu$g/mouse</th>
<th>MCD* $\mu$g/ml</th>
<th>MHD $\mu$g/rat</th>
<th>MND $\mu$g/rat</th>
<th>MCPID* $\mu$g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Htantabin</td>
<td>61-90</td>
<td>19</td>
<td>$4.63 \pm 2.63$</td>
<td>4.2</td>
<td>1.38</td>
<td>30.9</td>
<td>33.1</td>
<td>0.014</td>
</tr>
<tr>
<td>91-120</td>
<td></td>
<td>15</td>
<td>$3.26 \pm 2.78$</td>
<td>1.8</td>
<td>0.98</td>
<td>35.1</td>
<td>21.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Kungyankone</td>
<td>61-90</td>
<td>27</td>
<td>$6.05 \pm 0.98$</td>
<td>5.1</td>
<td>0.31</td>
<td>26.3</td>
<td>23.4</td>
<td>0.115</td>
</tr>
<tr>
<td>91-110</td>
<td></td>
<td>25</td>
<td>$8.74 \pm 3.94$</td>
<td>1.8</td>
<td>0.63</td>
<td>32.4</td>
<td>20.4</td>
<td>0.033</td>
</tr>
<tr>
<td>Hmawbi</td>
<td>61-90</td>
<td>7</td>
<td>$5.76 \pm 3.23$</td>
<td>6.0</td>
<td>0.12</td>
<td>56.9</td>
<td>52.5</td>
<td>0.063</td>
</tr>
<tr>
<td>91-110</td>
<td></td>
<td>8</td>
<td>$5.18 \pm 2.23$</td>
<td>6.0</td>
<td>0.34</td>
<td>52.5</td>
<td>40.7</td>
<td>0.037</td>
</tr>
<tr>
<td>Hlegu</td>
<td>61-90</td>
<td>6</td>
<td>$5.47 \pm 1.94$</td>
<td>4.2</td>
<td>0.93</td>
<td>37.2</td>
<td>23.4</td>
<td>0.050</td>
</tr>
<tr>
<td>91-110</td>
<td></td>
<td>6</td>
<td>$9.31 \pm 2.65$</td>
<td>6.3</td>
<td>0.48</td>
<td>32.7</td>
<td>50.1</td>
<td>0.030</td>
</tr>
<tr>
<td>Indaing</td>
<td>61-90</td>
<td>20</td>
<td>$6.38 \pm 0.98$</td>
<td>6.3</td>
<td>0.54</td>
<td>49.0</td>
<td>67.6</td>
<td>0.032</td>
</tr>
<tr>
<td>91-120</td>
<td></td>
<td>17</td>
<td>$4.97 \pm 3.7$</td>
<td>5.1</td>
<td>0.47</td>
<td>44.9</td>
<td>56.9</td>
<td>0.025</td>
</tr>
</tbody>
</table>

$f = 95\%$ fiducial limit
Results are mean of duplicates and triplicates*

### Table 6. Biological properties of venoms of young Russell’s vipers

<table>
<thead>
<tr>
<th>Property</th>
<th>Lower Myanmar</th>
<th>Upper Myanmar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ayerwaddy n=3</td>
<td>Yangon n=5</td>
</tr>
<tr>
<td>LD$_{50}$ $\mu$g/mouse</td>
<td>Nyaungdon 2.87</td>
<td>Htantabin 4.63</td>
</tr>
<tr>
<td>MHD $\mu$g/rat</td>
<td>Danuphyu 30.9</td>
<td>Htantabin 30.9</td>
</tr>
<tr>
<td>MND $\mu$g/mouse</td>
<td>Daedaye 29.85</td>
<td>Kungyankone 20.4</td>
</tr>
<tr>
<td>MDD $\mu$g/mouse</td>
<td>Nyaungdon 2.4</td>
<td>Kungyankone 1.8</td>
</tr>
<tr>
<td>MCD $\mu$g/ml</td>
<td>Nyaungdo 0.1</td>
<td>Hmawbi 0.12</td>
</tr>
<tr>
<td>MCPID $\mu$g/ml</td>
<td>Danuphyu 0.004</td>
<td>Htantabin 0.014</td>
</tr>
</tbody>
</table>
It could be summarised that venoms of young vipers from lower Myanmar (Nyaungdon, Danuphyu, Htantabin, Kungyankone, and Tharawaddy) possess biological properties except coagulant (Kyauksae).

**Venom variation among adult Russell’s vipers**

Venom possessing potent biological properties from each division (Table 9); lower and upper Myanmar (Table 10) and five divisions (Table 8) are shown in respective tables.

**Table 7. Potent biological properties of venoms of young Russell’s vipers**

<table>
<thead>
<tr>
<th>Property</th>
<th>Lower Myanmar</th>
<th>Upper Myanmar</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{LD}_{50} ) µg/mouse</td>
<td>Nyaungdon 2.87</td>
<td>Thaze 6.3</td>
</tr>
<tr>
<td>MHD µg/rat</td>
<td>Danuphyu/Htantabin 30.9</td>
<td>Taungdwingyi 41.7</td>
</tr>
<tr>
<td>MND µg/rat</td>
<td>Kungyankone 20.4</td>
<td>Thaze 39.8</td>
</tr>
<tr>
<td>MDD µg/mouse</td>
<td>Kungyankone 1.8</td>
<td>Taungdwingyi 13.0</td>
</tr>
<tr>
<td>MCD µg/ml</td>
<td>Minhla 0.072</td>
<td>Kyauksae 0.04</td>
</tr>
<tr>
<td>MCPID µg/ml</td>
<td>Thayarwaddy 0.0007</td>
<td>Kyauksae 0.066</td>
</tr>
</tbody>
</table>

**Table 8. Comparison of the potent biological properties of venoms of young and adult Russell’s vipers**

<table>
<thead>
<tr>
<th>Property</th>
<th>Young viper</th>
<th>Adult viper</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{LD}_{50} ) µg/mouse</td>
<td>Nyaungdon 2.87</td>
<td>Daedaye 2.86</td>
</tr>
<tr>
<td>MHD µg/rat</td>
<td>Danuphyu 30.9</td>
<td>Aunglan 23.7</td>
</tr>
<tr>
<td>MND µg/rat</td>
<td>Kungyankone 20.4</td>
<td>Pantanaw 20.42</td>
</tr>
<tr>
<td>MDD µg/mouse</td>
<td>Kungyankone 1.8</td>
<td>Taungdwingyi 1.0</td>
</tr>
<tr>
<td>MCD mg/ml</td>
<td>Kyauksae 0.04</td>
<td>Nyaungdon 0.033</td>
</tr>
<tr>
<td>MCPID µg/ml</td>
<td>Thayarwaddy 0.0007</td>
<td>Tharawaddy 0.0007</td>
</tr>
</tbody>
</table>

It could be summarised that venoms of young vipers from lower Myanmar (Nyaungdon, Danuphyu, Htantabin, Kungyankone, and Tharawaddy) possess biological properties except coagulant (Kyauksae).

**Table 9. Biological properties of venoms of adult Russell’s vipers**

<table>
<thead>
<tr>
<th>Property</th>
<th>Lower Myanmar</th>
<th>Upper Myanmar</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{LD}_{50} ) µg/mouse</td>
<td>Daedaye 2.86</td>
<td>Thaze 3.75</td>
</tr>
<tr>
<td>MHD µg/rat</td>
<td>Pyapone 23.99</td>
<td>Kyausae 54.3</td>
</tr>
<tr>
<td>MND µg/rat</td>
<td>Pantanaw 20.42</td>
<td>Thawe 39.31</td>
</tr>
<tr>
<td>MDD µg/mouse</td>
<td>Daedaye 1.78</td>
<td>Thaw 2.0</td>
</tr>
<tr>
<td>MCD µg/ml</td>
<td>Nyaungdon 0.033</td>
<td>Thawe 0.630</td>
</tr>
<tr>
<td>MCPID µg/ml</td>
<td>Daedaye 0.009</td>
<td>Thawe 0.0707</td>
</tr>
</tbody>
</table>

Geographical Variation of Biological Properties of Russell’s Viper Venom
In summary, venoms of adult vipers from lower Myanmar (Daedaye, Pantanaw, Nyaungdon, Tharawaddy) possess potent lethal, necrotic, coagulant and capillary permeability increasing except defibrino-genating (Taungdwingyi) and haemorrhagic (Aunglan) activities. Adult Russell’s viper venom of Kyauksae is devoid of capillary permeability increasing activity and possesses poor defibrinogenating and lethal activities.

It is interesting to note that potent biological activities of the potent venoms of young vipers are comparable to that of adult (Table 8). In general venom of both young and adult vipers of lower Myanmar possess potent biological activities except defibrinogenating, haemorrhagic (adult) and coagulant (young). It is noteworthy that venoms of both young and adult vipers of Tharawaddy possess potent capillary permeability increasing activity among all.

**DISCUSSION**

Geographical variation in toxicity of venom has been reported (Minton and Weinstein, 1976, Glenn and Straight, 1978, Jayanthi and Gowda, 1988). Preliminary study of venom from four localities of Yangon showed length and geographical variation of biological properties of Russell’s viper (Daboia russelii siamensis) venom (Tun-Pe et al., 1993). Geographical variation of biological properties of Russell’s viper (Daboia russelii siamensis) venom from five divisions of Myanmar has been documented (Tun-Pe et al., 2000a). In clinical field practice, two population of snake (young and adult) were responsible for bites during harvesting season (Myint-Lwin et al., 1985, Sann-Mya et al., 1998, Kyaw-Than et al., 1997). Therefore, biological properties of venom were studied on two groups of snake; young (<90 cm) and adult (>90 cm) based on the observation that 96% (51/55) pregnant vipers have a total length of >90 cm (Tun-Pe et al., 1995).

Usually the colour of venom of young vipers is white to pale yellow and that of adult is yellow depending on its L amino acid oxidase content. White venom has less L amino acid oxidase enzyme content (Zeller, 1948). Ninety-two percent of venom of young vipers of Danuphyu is white compared to other young viper venoms (64%) showing geographic variation of the colour of the venom (Aye-Aye-Myint et al., 1997).

Venom from one locality of a division does not possess all potent biological activities and individual potent biological property is found in venoms from various divisions. These
variations in venom properties account for variation in clinical features of Russell's viper bite cases from different localities (Kyaw-Than et al., 1997, Myint-Lwin et al., 1985, Sann-Mya et al., 1998, Tun-Pe et al., 2000b) and also variable performance of a batch of antivenom in neutralizing biological properties of venoms collected from five localities (Tun-Pe et al., 1999).

Since potent biological properties of venom of young vipers from five divisions are comparable to that of adults (Table 8) both venoms could be used in raising antivenom for common use. Young viper venoms were found to be as potent as those of adults (Tun-Pe et al., 1995). It has been reported that an effective bite of a young viper produced a clinical picture comparable to that of an adult’s bite (Myint-Lwin et al., 1985, Sann-Mya et al., 1998).

Since the venom of both young and adult vipers of Tharawaddy possess potent capillary permeability increasing activity among all, (this property is mainly observed in our Russell’s viper bite cases only), it is suggested that these venoms should be included in the venom pool used for raising antivenom for common use (Kornalik and Taborska, 1985).

Venoms collected from lower Myanmar are found to possess potent biological activities, except defibrinogenating, haemorrhagic and coagulant. The venoms used for raising antivenoms by Myanmar Pharmaceutical Factory are mainly derived from pooling of locally available venoms from lower Myanmar. This may explain why 2-40 times more antivenom was needed to neutralize different biological activities of venom of Taungdwingyi (Upper Myanmar) compared to that of venom of Daedaye (Lower Myanmar) (Tun-Pe et al., 1994).

It is envisaged that widely pooled potent venoms will be ideal for raising antivenom for common use. These findings will be useful for antivenom manufacturers as well as clinicians or medical officers who are looking after snakebite patients throughout the country.

References
Management of Snakebite and Research


Neutralization of Biological Activities of Russell’s viper (Daboia russelii siamensis) Venom by Antivenoms

Tun-Pe, Aye-Aye-Myint, Kyi-May-Htwe and Nu-Nu-Aung

Venom Research Laboratory, Department of Medical Research, Yangon

Abstract

Feasibility of using antivenoms manufactured abroad in Myanmar Russell’s viper (D.r.siamensis) bites is investigated. Neutralization of different biological activities of Myanmar Russel’s viper venom by monospecific antivenom manufactured by Myanmar Pharmaceutical Factory (MPF), Thai Red Cross (TRC), polyspecific Indian SII and Barat serums were tested by WHO recommended methods. Two to four times the amount of TRC, 2-40 times of the Barat Serums and 2-128 times more of the SII antivenoms were needed to neutralize different biological properties of the Russell’s viper venom compared to reference and the SII antivenom failed to neutralize procoagulant activity of the venom. Five and 64 times the amount of the MPF antivenom are required to neutralize the necrotic and capillary permeability increasing activities of the venom.

It is concluded that local antivenom raised with local venom is best for Myanmar Russell’s viper bite cases, but batch-to-batch variation of potency of MPF antivenom should be monitored.

INTRODUCTION

Myanmar has an annual snakebite incidence of 5,227 bites in 1990, based on data collected from 114 hospitals (unpublished observation) with Russell’s viper (Daboia russelii siamensis) responsible for 60%, cobra (Naja kaouthia) 4%, green pit viper (Tr. spp.) 3.7%, sea snake 0.1% and unknown bites 32%. Most unknown bites are attributed to Russell’s viper and non-poisonous bites. Myanmar Pharmaceutical Factory (MPF) manufactures monospecific antivenom for treating Russell’s viper and cobra bites. Four ampoules of antivenom is recommended for treating systemic envenomed cases of Russell’s viper bites and eight ampoules for systemic envenomed cases with complications (National Seminar on Snakebite, 1989, Hla-Myint et al., 1982). Our earlier study indicated that 38% of Russell’s viper bites admitted to the Taungdwingyi hospital are dry bites (Sann-Mya et al., 1998). Chances of human exposure to venomous snakes increase with the introduction of multiple cropping. In order to meet the antivenom demand, increased local production of antivenom is recommended for treating systemic envenomed cases with complications (National Seminar on Snakebite, 1989, Hla-Myint et al., 1982). Our earlier study indicated that 38% of Russell’s viper bites admitted to the Taungdwingyi hospital are dry bites (Sann-Mya et al., 1998).
MATERIALS AND METHODS

Characterization of Russell’s viper venom

Pooled Russell’s viper venom collected from snakes (n14) (>90 cm) of Tharawaddy, Bago division was used in the experiments. Biological properties such as LD_{50,IV}, coagulant, necrotic, haemorrhagic, defibrinogenating and capillary permeability increasing (CPI) activities of the venom were determined by WHO recommended techniques (Theakston and Reid, 1983).

Neutralization of Russell’s viper venom by antivenom

A monospecific enzyme refined equine Russell’s viper antivenom batch F 96770, expiry, 2001 (MPI), Thai Red Cross (TRC) monospecific lyophilized refined equine antivenom (batch 4, exp. 9/001), poly specific lyophilized equine antivenom from Serum Institute (SII) of India (batch 109, exp. 4/96) and poly specific liquid ASV from Barat Serums and vaccines, India (batch KD2) were tested for neutralization of different biological properties of the Russell’s viper venom, such as lethality (LD_{50,iv}), coagulant (10MCD), haemorrhagic (3MHD), necrotic (3MND), defibrinogenating (5MDD) and capillary permeability increasing (100 MCPID) activities using WHO recommended standard test of neutralizing activity (Theakston and Reid., 1983). A lyophilized mono-specific enzyme refined equine Russell’s viper antivenom of MPI (batch DH95758 exp. 11/001) serves as a reference.

Briefly, a fixed amount of venom is mixed with a variable dilution of antivenom, incubated at 37°C for 30 min. and injected into laboratory animals. The end point is taken as the minimum amount of antivenom required to neutralize 50% of the biological effects of venom. For determination of neutralization of lethal activity, ED_{50} (median effective dose) was used. It is the minimum amount of antivenom that will save 50% of the test animals in 24 hours following the injection.

RESULTS

Potency assay of Russell’s viper antivenoms

Results of neutralization of the biological properties of the Russell’s viper venom by the antivenoms are shown in the table 1.

<table>
<thead>
<tr>
<th>Antivenom</th>
<th>3MHD (mg/ml)</th>
<th>3MND (mg/ml)</th>
<th>5MDD (µg/ml)</th>
<th>100MCPID (µg/ml)</th>
<th>LD_{50,iv} (µg/ml)</th>
<th>10MCD (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>10*</td>
<td>5*</td>
<td>3*</td>
<td>0.078*</td>
<td>5*</td>
<td>0.0625*</td>
</tr>
<tr>
<td>MPI</td>
<td>12.5</td>
<td>25</td>
<td>2.5</td>
<td>5</td>
<td>5</td>
<td>0.078</td>
</tr>
<tr>
<td>TRC</td>
<td>10</td>
<td>20</td>
<td>5</td>
<td>0.3125</td>
<td>5</td>
<td>0.125</td>
</tr>
<tr>
<td>Barat</td>
<td>37.5</td>
<td>37.5</td>
<td>2.5</td>
<td>1.25</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>SII</td>
<td>400</td>
<td>400</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Figures are µl of antivenom; Results are mean of duplicates; ND= not neutralized; LD_{50,iv}= lethality; MDD = minimum defibrinogenating dose; MCD = minimum coagulant dose; MHD = minimum haemorrhagic dose; MND = minimum necrotic dose; MCPID = minimum capillary permeability increasing dose; MPI = Myanmar Pharmaceutical Factory; TRC=Thai Red Cross; SII = Serum Institute of India; Barat =Barat serums and vaccines.
Neutralization of Biological Activities of Russell’s viper (Daboia russelii siamensis) Venom by Antivenoms

It is observed that 2-4 times amount of TRC antivenom are required to neutralize, coagulant, defibrinogenating, necrotic and capillary permeability increasing (CPI) activities and is equally effective in neutralizing lethal and haemorrhagic properties of the venom where as 2-80 times of SII is required to neutralize the different biological properties of the venom except CPI activity (128 times). It fails to neutralize the coagulant property of the venom. Two to 40 times amount of Barat Serums are required to neutralize lethal, haemorrhagic, necrotic, CPI and coagulant activities of the venom. Five and 64 times amount of MPF antivenom are required to neutralize necrotic and CPI activities of the venom.

DISCUSSION

The present study indicates that antivenom prepared with local venom achieved a better performance in neutralizing biological activities of the venom. The differences observed in neutralizing activities of the antivenoms could be attributed to geographical variation of the venom (Jayanthi and Gowda, 1988) and species differences of Russell’s viper venom used in raising the antivenoms. Geographical variation of the clinical features of Russell’s viper bite cases from Myanmar, Thai and India (Warrell, 1986), failure of neutralization of venom induced effects produced by Ceylonese Russell’s viper by Indian Haffkine (V. r. puchella) antivenom (Phillips et al., 1988) and venom neutralizing efficacy of the antivenoms (1ml reference antivenom will neutralize 2mg, MPF (1mg), SII, Barat Serums (0.6mg) and TRC (0.4mg) of the respective venoms have been documented. Batch to batch variations of venom used for raising MPF antivenom (Tun-Pe et al., 1996a) and variable performance of the antivenoms (Tun-Pe et al., 1996 b) could account for variation in performance of MPF antivenom.

Venom neutralising efficacy of SII antivenom (India) is much inferior to the Barat Serums (India) and will require a large volume of antivenom to neutralize different biological activities of the venom which carries risk of development of hypersensitivity reactions. Moreover the former failed to neutralize the procoagulant activity of the venom where the latter required 40 times amount of antivenom to achieve it. However, TRC antivenom that required 2-4 time amount of the antivenom to neutralize the venom properties could be an alternative choice to the MPF. But antivenom prepared with local venom is best for treating snakebite cases from the same locality (Phillips et al., 1988) has been documented.

It is suggested that the quantity of Russell’s viper antivenom manufactured by MPF could be made sufficient if proper clinical assessment of Russell’s viper bite cases is carried out by the medical officers instead of giving antivenom routinely to all snakebite cases. Continuing education of medical officers on updated management of snakebite based on research findings is needed.

References


Neutralization of Biological Activities of Russell’s Viper (*Daboia russelii siamensis*) venoms from different localities of Myanmar by monospecific Antivenom

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Venom Research Laboratory, Department of Medical Research, Yangon

**Abstract**

Neutralization of biological properties of Russell’s viper venoms from five divisions of Myanmar, namely Mandalay, Magwe (Upper Myanmar), Yangon, Bago, Ayerawaddy (Lower Myanmar) by a mono-specific antivenom was studied by WHO recommended methods of neutralization tests in rodents. In general, 2-8 times more antivenom is required to neutralize different biological activities of the venom of Upper Myanmar compared to the Lower. Forty times more antivenom is needed to neutralize procoagulant activity of the venom of Taungdwingyi (Magwe division) compared to that of Daedaye (Ayerawaddy division). The antivenom is equally effective in neutralizing procoagulant activity of the venom of Wundwin (Mandalay division). Variable neutralization of the venom properties by the antivenom among venoms of Lower Myanmar is probably due to intradivisional variation in venom properties. The observation calls for use of widely pooled venoms in raising antivenom for common use.

**INTRODUCTION**

Russell’s viper (*Daboia russelii siamensis*) bite occurs in the rice growing divisions of Myanmar. Monospecific antivenom manufactured by Myanmar Pharmaceutical Factory (MPF) is used for treating Russell’s viper bite cases throughout Myanmar. The source of venom used for raising antivenom is mainly derived from venoms collected from Russell’s vipers caught in Yangon, Bago and Ayerawaddy divisions (Lower Myanmar) which tend to vary from year to year. However, recent observations of geographical variation of Russell’s viper venom (Aye-Aye-Myint et al., 1993, 1994, 1995, Sann-Mya et al., 1994, Tun-Pe et al., 1993); variation of venom neutralizing efficacy of a batch of mono specific antivenom against venoms from three different localities of Myanmar (Tun-Pe et al., 1994) and variable performance of antivenom in correcting coagulation defect in Russell’s viper bite cases of different localities of Myanmar (Kyaw-Than et al., 1997, Myint-Lwin et al., 1983, Sann-Mya et al., 1998, Tun-Pe et al., 2000a) prompted us to do this study.
MATERIALS AND METHODS

Characterization of the venoms

Russell’s vipers caught from five divisions of Myanmar namely Mandalay, Magwe (Upper Myanmar), Yangon, Bago, Ayerawaddy (Lower Myanmar) were housed separately and kept in the snake farm of MPF. Venom milked from individual snake was lyophilized and pooled according to locality and length into young (<90cm) and adult (>90 cm). Pooled venom of adult vipers of Mandalay division (Wundwin n=2), Magwe (Taungdwingyi n=4), Yangon (Kungyankone n=25), Bago (Tharawaddy n=14) and Ayerawaddy (Daedaye n=7) were stored in dark at 4°C. (Tun-Pe et al., 2000 b). Characterization of the biological properties of the venoms such as lethal (LD$_{50iv}$), coagulant (MCD), defibrinogenating (MDD), necrotic (MND), haemorrhagic (MHD) and capillary permeability increasing (MCPID) was determined according to WHO recommended methods (Theakston and Reid, 1983). Lyophilized antivenom was reconstituted, aliquoted and stored frozen at -20°C until tested.

Venom-antivenom neutralization test

Monospecific antivenom, batch no DN 86608B exp. 4/92 was used for neutralization test in rodent according to WHO standard tests of neutralizing activity (Theakston and Reid, 1983). For venom-antivenom neutralization test, 5LD$_{50iv}$, 10MCD, 3MHD, 3MND, 5MDD and 100 MCPID doses were used. Briefly, a fixed amount of venom is mixed with a variable dilution of antivenom, mixed, incubated at 37°C for 30 min. and then injected into the laboratory animals. The end point is taken as the minimum amount of antivenom required to neutralize 50% of the biological effects of venom. For determination of neutralization of lethal activity, ED$_{50}$ (median effective dose) was used. It is the minimum amount of antivenom that will save 50% of the test animals in 24 hours following the injection. (Theakston and Reid, 1983).

RESULTS

Venom doses used for neutralization of the antivenom are shown in Table 1.

<table>
<thead>
<tr>
<th>Source of venom</th>
<th>5LD$_{50iv}$ µg/mouse</th>
<th>10MCD µg/ml</th>
<th>3MHD µg/rat</th>
<th>3MND µg/rat</th>
<th>5 MDD µg/mouse</th>
<th>100 MCPID µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandalay division (Wundwin)</td>
<td>16.9</td>
<td>31.62</td>
<td>135.2</td>
<td>112.5</td>
<td>15.0</td>
<td>0.295</td>
</tr>
<tr>
<td>Magwe division (Taungdwingyi)</td>
<td>19.9</td>
<td>50.12</td>
<td>92.7</td>
<td>97.1</td>
<td>6.0</td>
<td>0.832</td>
</tr>
<tr>
<td>Bago division (Tharawaddy)</td>
<td>23.4</td>
<td>25.12</td>
<td>126.5</td>
<td>119.4</td>
<td>12.0</td>
<td>0.724</td>
</tr>
<tr>
<td>Yangon division (Kungyankone)</td>
<td>30.2</td>
<td>31.25</td>
<td>79.0</td>
<td>70.3</td>
<td>25.5</td>
<td>0.115</td>
</tr>
<tr>
<td>Ayerawaddy division (Daedaye)</td>
<td>14.3</td>
<td>1.35</td>
<td>84.5</td>
<td>83.6</td>
<td>8.9</td>
<td>0.892</td>
</tr>
</tbody>
</table>

LD$_{50iv}$ = lethality, MCD = minimum coagulant dose, MND = minimum necrotic dose, MDD = minimum defibrinogenating dose, MHD = minimum haemorrhagic dose, MCPID = minimum capillary permeability increasing dose.

Table 1. Venom doses used for venom-antivenom neutralization test
Results of venom neutralizing dose of the antivenom with venoms from five different divisions of Myanmar are shown in Table 2.

Table 2. Amount of antivenom required for neutralizing biological properties of venoms from different localities of Myanmar

<table>
<thead>
<tr>
<th>Source of Venom</th>
<th>5LD_{50} iv µl</th>
<th>10 MCD µl</th>
<th>3 MND µl</th>
<th>3 MDD µl</th>
<th>100 MCPID µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magwe division</td>
<td>10</td>
<td>2.5</td>
<td>20</td>
<td>20</td>
<td>1.25</td>
</tr>
<tr>
<td>Taungdwingyi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandalay division</td>
<td>10</td>
<td>0.078</td>
<td>20</td>
<td>20</td>
<td>1.25</td>
</tr>
<tr>
<td>Wundwin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yangon division</td>
<td>5.0</td>
<td>0.0625</td>
<td>0.0625</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Kungyankone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bago division</td>
<td>2.5</td>
<td>0.0625</td>
<td>2.5</td>
<td>2.5</td>
<td>0.625</td>
</tr>
<tr>
<td>Tharawaddy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ayerawaddy division</td>
<td>2.5</td>
<td>0.0625</td>
<td>2.5</td>
<td>2.5</td>
<td>0.625</td>
</tr>
<tr>
<td>Daedaye</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LD_{50} iv = lethality; MCD = minimum coagulant dose; MND = minimum necrotic dose; MDD = minimum defibrinogenating dose; MCPID = minimum capillary permeability increasing dose.

It is observed that in general, 2-8 times more antivenom is needed to neutralize different biological properties of the venoms of Upper Myanmar (Magwe and Mandalay) compared to Lower Myanmar (Tharawaddy, Kungyankone and Daedaye). Forty times more antivenom is needed to neutralize procoagulant activity of the venom of Taungdwingyi (Magwe) compared to the Daedaye (Ayerawaddy). However, it is equally effective in neutralizing procoagulant activity of the venom of Wundwin (Mandalay). Two to four times more antivenom is needed to neutralize lethal and defibrinogenating and 2-4 times less for necrotic and capillary permeability increasing activities of the venom of Kungyankone (Yangon) are observed on comparing venom neutralizing efficacy of the antivenom among the venoms of Kungyankone (Yangon), Tharawaddy (Bago) and Daedaye (Ayerawaddy) (Lower Myanmar).

DISCUSSION

The study highlighted that more antivenom is needed to neutralize biological activities of venoms of upper Myanmar (Taungdwingyi and Wundwin) compared to lower Myanmar (Tharawaddy, Kungyankone and Daedaye). It has been reported that more antivenom was needed to neutralize biological activities of Taungdwingyi venom compared to Daedaye (Tun-Pe et al., 1994). Since venoms used for raising antivenom for common use are derived from lower Myanmar, a better neutralization with the venoms from lower Myanmar is observed in this study. Antivenom raised with local venom is good for use in that locality was observed by Phillips et al (1988) where Indian Haffkine antivenom failed to correct coagulation defect produced by bites of Ceylonese Russell’s viper (V. pachella) and up to 530 ml of the antivenom was proved to be necessary for restoring haemostatic defect. Jayanthi and Gowda (1988) indicated that there was lack of protection afforded in Russell’s
viper bite victims from southern India when given Haffkine antivenom. All observations called for use of antivenom raised with local venoms, if possible local antivenom raised with venoms from upper or lower Myanmar or common antivenom raised with widely pooled venoms from different localities since there is geographical variation of composition of venom (Tun-Pe et al., 2000).

References


Potency Assay of the Liquid Russell’s Viper (Daboia russelii siamensis) Antivenom Stored at Different Environment

Tun-Pe, Aye-Aye-Myint and Kyi-May-Htwe
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Abstract
The efficacy of liquid Russell’s viper antivenom stored at two different conditions: (pot/ground) keeping it in a sand pot embedded in the ground and another (pot/house) in a sand pot embedded in a larger sand pot and kept in the house with two schedules of watering sand, daily and every third day was studied for seven months covering the winter and hot summer months in two localities in midland, Taungoo and Taungdwingyi respectively. At the end of the study the efficacy of antivenoms were assessed by WHO recommended methods. Daily watering keeps the sand temperature 1-2ºC cooler than every third day and maintains its temperature at 30-32ºC during the hot summer days of 40ºC. The efficacy of the antivenom in pot-ground with daily watering was found to be comparable to the control and superior than pot-ground with every third day watering and also to that stored in the pot-house with daily watering. It is suggested that liquid antivenom should be stored in a sand pot embedded in the ground with daily watering of the sand in places where no cold storage facility is available. It is simple, effective, in expensive and could be carried out by the rural farmers.

INTRODUCTION
Snakebite is an occupational hazard of our rural farmers. Early administration of potent antivenom following the bite plays a vital role in the management of snakebite. Early administration of antivenom combined with local compression immobilization first aid is found to be effective in delaying onset of systemic envenoming (Tun-Pe et al., 1998). In Taungdwingyi district, 15% of the snakebite cases received 1-2 ampoules of either liquid or lyophilized antivenom following the bite in the villages (Tun-Pe et al., 1998). However, because of lack of electricity, expensive antivenoms are either kept at room temperature or in wet sand in order to keep them cool. It has been shown that liquid antivenom if store at 37ºC for six months, begins to lose its efficacy (Christensen, 1975). It is desirable that expensive antivenom should be stored at an optimal environment in rural areas in order to preserve its efficacy. This experiment attempts to find out the optimal environment for storage of liquid antivenom with minimal loss of its efficacy in rural snakebite endemic areas.
MATERIALS AND METHODS

A batch of liquid monospecific Russell’s viper antivenom, batch no. F96770, expiry 7/99 manufactured by Myanmar Pharmaceutical Factory was used in the experiment. Antivenom stored at 4°C in the Venom Research Laboratory, Department of Medical Research served as control.

Since environment temperature of midland is high and variable, two sites (Taungdwingyi and Taungoo) were chosen for the study. Because of lack of security of storage of antivenom in the ground in Taungdwingyi, two different methods of storing antivenom were carried out at the two sites.

In Taungoo, (pot/ground) the anti-venom wrapped with a piece of polythene sheet is embedded in sand filled earthen porous pot of 30 cm in diameter. The pot was embedded in the ground to its neck in the shade with its lid on. Ten mm thick sand was layered between the pot and the ground (Figure 1). A similar experiment was set up at a distance of 45 cm. Two sets of experiments, daily (schedule A) and every third day (schedule B) watering of the sand were carried out. Two or three glasses of water were used to water the sand inside and the outside of the pot respectively. The temperature of the sand inside the pot and the shade temperature were recorded at noon daily before watering.

In Taungdwingyi (pot/house), a similar size sand pot containing the antivenom as in pot-ground method was embedded in larger sand filled pot that was kept inside the house (Figure 2). The same schedule of watering the sand and recording the temperature was followed. The experiments lasted for seven months in Taungoo and six months in Taungdwingyi, which covered the winter and the hot summer months of the year.

At the end of the study, the efficacy of the antivenoms was assessed by testing neutralization of different biological properties of Russell’s viper venom such as lethality.
(LD<sub>50</sub> IV.), coagulant (10MCD), haemorrhagic (3MHD), necrotic (3MND), defibrinogenating (5MDD) and capillary permeability increasing (100MCPID) activities by the antivenoms using WHO recommended standard test of neutralizing activity (Theakston and Reid, 1983).

Briefly, a fixed amount of venom is mixed with a variable dilution of antivenom, incubated at 37°C for 30 min. and injected into laboratory animals. The end point is taken as the minimum amount of antivenom required to neutralize 50% of the biological effects of venom. For determination of neutralization of lethal activity ED<sub>50</sub> (median effective dose) is used. It is the minimal amount of antivenom that will save 50% of the test animals. SDS-PAGE electrophoresis, immunoblotting and immunostaining of the venom with the antivenoms were carried out according to the methods already described (Lamelii, 1970, Towbin et al., 1979).

RESULTS

Results of neutralization of the different biological properties of the venom by the antivenoms are shown in Table 1.

Table 1. Amount of antivenom required for neutralizing different biological properties of the Russell’s viper venom

<table>
<thead>
<tr>
<th>Method/antivenom</th>
<th>5LD&lt;sub&gt;50&lt;/sub&gt; µg/ml</th>
<th>3MHD mg/ml</th>
<th>3 MND mg/ml</th>
<th>10MCD 50.25 µg/ml</th>
<th>5MDD 120 µg/ml</th>
<th>100 MCPID 1.48 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5*</td>
<td>1.25*</td>
<td>2.5*</td>
<td>0.0781*</td>
<td>2.5*</td>
<td>5*</td>
</tr>
<tr>
<td>Pot/grd schedule A</td>
<td>10</td>
<td>2.5</td>
<td>2.5</td>
<td>0.0781</td>
<td>2.5</td>
<td>1.25</td>
</tr>
<tr>
<td>Pot/grd schedule B</td>
<td>10</td>
<td>5</td>
<td>2.0</td>
<td>0.1562</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Pot/house schedule A</td>
<td>5</td>
<td>1.25</td>
<td>5</td>
<td>0.3125</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>Pot/house schedule B</td>
<td>5</td>
<td>1.25</td>
<td>2.5</td>
<td>0.0781</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

* = µl

Control = the same batch of antivenom stored at 4°C.
Pot/grd = antivenom of pot/ground.
Pot/house = antivenom of pot/house.
Schedule A = daily watering of the sand.
Schedule B = every third day watering of the sand.

LD<sub>50</sub> IV = lethality,
MDD = minimum defibrinogenating dose,
MCD = minimum coagulant dose,
MHD = minimum haemorrhagic dose,
MND = minimum necrotic dose,
MCPID = minimum capillary permeability increasing dose.

In pot/ground method, the neutralizing efficacy of the antivenom with daily watering (Schedule A) was comparable to the control (except in neutralization of lethal and haemorrhagic) and has a better performance than the antivenom with every third day watering (Schedule B).

In pot/house method, the neutralizing efficacy of the antivenom with daily watering (Schedule A) was inferior to that of the antivenom with every third day watering (Schedule B).
A better preservation of antivenom efficacy was observed in pot/ground with daily watering than pot/house with daily watering.

Immunoblotting of the venom with the antivenoms showed there were no quantitative differences in recognizing bands by the antivenoms (Figure 3).

**Figure 3. Picture of immunoblot of the Russell’s viper venom with the antivenoms under studied**

The relationship between the temperature of the sand inside the pot and shade/room temperature is shown in Table 2.

Daily watering of the sand keeps the sand temperature 1-2°C cooler than every third day watering and could maintain temperature of the sand at 30-32°C (8-10°C lower) when the shade/room temperature reaches 40°C during the hot summer months.

**Table 2. Relationship between temperature of the sand inside the pot and shade/room temperature in the two study sites**
DISCUSSION

The study highlighted that the potency of the liquid antivenom stored in a sand pot embedded in the ground (pot/ground) with daily watering of the sand is maintained for seven months covering the hottest summer days of the midland. Daily watering of the sand has an advantage over the every third day in keeping the sand temperature 1-2°C cooler and it could be carried out routinely at the villages.

Enzyme refined antitoxin are more stable than the whole sera preparation and deterioration at temperature between 0 and 5°C is negligible (British Pharmacopia, 1969). At 37°C the preparation may lose 10-20% of their activity in one year. A slight reduction in neutralizing efficacy to different biological activities observed in our antivenoms could be due to storage of the products at a temperature well above 4°C. Liquid antivenom stored at 37°C for six months starts to precipitate out indicating loss of efficacy (Christensen, 1975). However, the maximal temperature of the sand recorded in the study is 32°C. In order to reach a storage temperature of 37°C the shade/room temperature should be 45-47°C that is unlikely, but could not be ruled out especially during summer months in the midland. Even then, the maximal temperature will not last for six months.

Although venom neutralizing efficacy of the antivenom in pot/house with daily watering is slightly inferior to pot/ground with daily watering, it could be used if the latter method of storage is not feasible as in our case.

It is concluded that liquid antivenom stored in a sand pot embedded in the ground with daily watering should be recommended for storing liquid and lyophilized antivenoms in snakebite endemic areas with no cold storage facility. Moreover it is simple, inexpensive, effective and could be carried out properly in rural villages without much effort. However, practice of rapid turnover of the fresh stock of liquid antivenom especially during the summer months or use of lyophilized preparation should also be encouraged (Tun-Pe et al., 1994).

References


INTRODUCTION

Russell’s viper (Daboia russelii siamensis) bite has been a serious medical problem in Myanmar for many decades. Post-bite administration of antivenom, a product of Myanmar Pharmaceutical Factory (MPF), is the only effective and widely used treatment. However, the mortality rate remains considerably high. On many occasions, antivenom treatment is late and irreversible damage of vital organs has already occurred. Also sometimes, the development of lesion is so rapid that the antivenom alone cannot neutralize the lethal effect where large amounts of venom are already introduced into the body. Therefore, development of a suitable and effective measure i.e. active immunization of people susceptible to snakebites is of utmost importance as a protective measure. If the victims are immune to the venom, even to a small degree, the onset of damage is delayed and they can be treated more successfully (WHO, 1981). Therefore, many attempts have been made by various scientists to develop of RVV toxoid for nearly three decades in Myanmar.
Conventional RVV toxoid

In 1969, Ko Ko Gyi et al tried to prepare RVV toxoid from desiccated RVV in the veterinary Department of BPI (now known as MPF), Yangon. RVV was dissolved in one percent phosphate buffer saline solution (pH 7.2) containing one percent peptone, to give 1 mg/ml concentration. Formalin was added to give 0.5 percent concentration and incubated at 37°C for four weeks. The toxicity of the detoxified venom was reduced to 60 times and 100 times over that of the initial venom when tested with rabbit and mouse respectively. Besides, rabbits inoculated intraperitonally with 5 ml of detoxified venom at three days’ interval for four times were found to be able to withstand 2-3 times the minimal lethal dose of venom when challenged seven days after the last immunizing dose. The results presented herein demonstrate that it is possible to detoxify RVV with formalin without effecting its immunogenicity. However, further detailed investigations of the toxoid regarding its stability, the level and persistence of circulating antibody, chronic toxicity of the toxoid on vital organs following immunization could not be carried out.

Refined RVV toxoid

In 1986, the Department of Medical Research (DMR) working group on RVV toxoid, successfully developed a refined RVV toxoid. The desiccated pooled crude RVV was fractionated using sephadex G50 fine with 0.01mol/l Tris-HCL buffer pH 7.5. The first four fractions obtained were pooled and concentrated and a mild and step wise formalin toxoiding was carried out by using a modified method of Kondo et al (1971).

This preparation was found to be safe, since mice tolerated 0.5ml of toxoid (i.e. more than 60 LD50). The antibody titers of mice and rabbits, which were immunized with toxoid subcutaneously for three booster injections at weekly interval commencing three weeks after the first injection, showed a rise of 1.2 gm % to 4 gm % in their sera, as shown by quantitative reversed rocket immunoelectrophoresis method. The immunized rabbit could withstand the challenge of 8 mg to 16 mg of RVV by intramuscular injection. The antibody titer estimated by the in vivo venom neutralization test revealed that 0.4 ml serum neutralized 200 to 300 mg of venom.

Monkeys (Macaca mulata) were immunized with an initial subcutaneous injection of 0.5 ml (containing 1 mg protein) by two boosters of same dosage given at four week intervals. Satisfactory rise of circulating antibody levels was seen in these monkeys and their rise persisted for 24 weeks. The immunized monkeys withstood intramuscular challenged with 2 mg/kg of crude venom (i.e. lethal dose) at two weeks after a course of toxoid injection. It also showed minimum undesirable local side effect with no detectable systemic adverse effect.

In another experiment, refined toxoid was given to 12 human volunteers. No abnormalities such as bleeding time, clotting time, haemoglobin concentration, total differential white cell count, serum bilirubin, alkaline phosphates, alanine and aspartate transferase, urinalysis and ECG done before injection and 2, 7 and 21 days after injection of refine toxoid were detected in the laboratory test. Significant rise of antibody titer was
detected in all subjects and persisted for 24 weeks. The antibody levels achieved in these human subjects were equivalent to levels seen in immunized monkeys which withstood the challenge with lethal dose of 2 mg/kg RVV. However, the practical problem of delivering immunization to the large population at risk is that this refine toxoid could not be manufactured on a large scale since this fractionation procedure yielded a very small amount of venom that could be further used for toxoidation.

**Crude RVV toxoid**

DMR also successfully developed a crude RVV toxoid directly prepared from crude RVV by slow and step wise formalinization method of Aung-Khin et al (1980). Rhesus (Macaca mulata) monkeys were used to study the effect of crude RVV toxoid on their kidneys, coagulation system and immunogenic response. It was found that there was no undesirable effect in those injected with toxoid (DMR, 1968).

**Studies on immunization schedule**

The influence of immunization schedule and age of the toxoid on antibody response in animals were studied on rabbits and monkeys by Aye-Aye-Myint and her group and the findings were reported in the proceedings. (Aye-Aye-Myint et al., 1996, 1997).

**Effects of storage on toxoid**

The formalin treated crude RVV toxoid stored at different storage temperature showed reversion of formaldehyde linkages with appearances of toxicity, reduction in immunogenicity and biological properties. Aye-Kyaw et al (1994) had showed that a significant amount of free formaldehyde start to appear after one month when stored at -20°C and after three months when stored at 4°C and 18°C.

Tun-Pe et al (1995) showed that toxoid stored at 4°C for four months showed reduction in lethal activity (15 fold LD50), coagulant activity (4 fold MCD), defibrinogenating activity (22 fold MDD), haemorrhage activity (6 fold MHD), necrotic activity (10 fold MND) and capillary permeability increasing activity (44 fold). Decrease in number of precipitin bands was observed when the toxoid aged. Such variation in biological properties of stored toxoid will influence the quality of antibody produced and the protective immunity of the immunized subjects.

Khin-Pa-Pa-Kyaw et al (1994) also reported that although RVV toxoid stored at 4°C remain non-toxic and immunogenic upto 12 months, those stored at room temperature and at 20°C became toxic from three months onwards and significantly reduced in immunogenicity from nine months onwards. Electrophoretic studies revealed that low molecular weight protein bands reappeared in the toxoid stored at room temperature and 20°C but not in toxoid stored at 4°C.
Study on improvement of toxidation methods and its stability

Many attempts have been made by the DMR working group on RVV toxoid research on the toxidation methods and stability of the formal toxoid regarding both safety and immunogenicity. Khin-Maung-Maung et al (1996) fractionate of crude RVV with sephadex G 75 super fine gel filtration chromatography and pooled the first four fractions which were further treated under slow and step wise formalinization. The fractionated RVV toxoid obtained showed no reversion in toxicity and also preserved immunogenicity up to six months storage at room temperature as well as storage at 4°C. However, electrophoretic study of the toxoid revealed that the low molecular weight protein bands reappeared in the toxoid stored at room temperature for three months and above, probably indicating the re- dissociation of polymerized protein during storage at room temperature.

San-Aye et al (1997) studied sodium bisulfite for preservation of the formaldehyde linkage in the toxoid. Thirty-five percent sodium bisulfite was added into the formalinized crude RVV toxoid during its preparation. In another experiment, RVV toxoid was prepared by adding formalin upto 2% concentration and sodium bisulfite was added upto 35%. Both of these two preparations of toxoid were stored at 4°C and 37°C for six months and tested for safety and immunogenicity. Both the experiments showed that toxoid stored at 37°C and 4°C were safe and immunogenic. However, the immunogenicity of toxoid with sodium bisulfite stored at 37°C is less than the toxoid at 4°C.

In 1999 Win-Aung et al lyophilized the liquid form of RVV toxoid and stored at 4°C and room temperature. It was apparent that lyophilized toxoid stored at both conditions showed minimal changes in activities of enzymes, less reversion in formalin linkages, more immunogenic and no change in toxicity when compared with those of liquid form of RVV toxoid. However, there was considerable amount of protein loss in lyophilized toxoid during the process of freeze drying. This will be obstacle in producing large amount of lyophilized toxoid for human immunization.

Recently, five major enzymatic components of RVV, namely phospholipase A2, phosphodiesterase, arginase esterase, coagulase and phosphomonoesterase were purified from crude RVV by using an appropriate gel filtration and or ion-exchange chromatographic columns and the formalinized RVV toxoid against these enzymes were then developed. This toxoid also showed no toxicity and also induced satisfactory immune responses in experimental mice compared to those of a conventional toxoid. However, when the fully immunized mice were challenged with the 3 LD50 i.e. lethal dose RVV, they could not totally withstand and died just after challenging (San-Aye, personal communication).

CONCLUSION

Generally, snake venoms are highly complex mixtures of various enzymes and toxic elements. Most of the constituents are proteins but low molecular weight compounds such as peptides, nucleotides and metal ions are also present (Karlsson, 1973). Therefore, toxidation of the snake venoms is very difficult as compared to that of single toxin like a bacterial toxin. In addition, because of the antigenic complexity of the venom proteins and their great local and systemic effects, active immunization against venom for the
production of commercial antivenom. It was also reported that immunity against the effect of snakebite did not exist for a long time among 14 human subjects, each of whom had been bitten twice or more by crotalid snakes (Parrish et al., 1959). Although active immunization of humans has been attempted with some success in other countries, i.e. toxoids against Indian cobra (Naja naja) (Flowers, 1963), Australian tiger snake (Notechis scutatus) (Weiner, 1961) and Japanese Habu snake (Trimeresurus flavoviridis) (Sawai et al., 1960), it was also reported that these toxoids did not provide real protection due mainly to the short lived antibody titres conferred; immunization would not work unless the challenge bite occurred soon after immunization procedure (Rosenfeld, 1971). However, the stability and effectiveness of these toxoids on long term storage had not been properly mentioned.

Based on the above literature review, it may be possible to immunize persons against Russell’s viper bite but those persons at risk may encounter many other species of snakes like cobra, krait, etc. Besides, it is necessary to administer frequent boosting of the subjects i.e. about every six weeks to obtain an adequate and protectable quantity of venom antibody persistently in the body at the time of bite to achieve a definite protection. Moreover, little is known on how humans would react to such untoward effects of the detoxified venom (toxoid) and its chronic toxicity on the vital organs of the body. The conclusion is that active immunization against RVV would be of limited value even if relatively save, effective and stable RVV toxoid could be produced by a simple toxoidation method. Therefore, any attempts to carry out further research on improvement of the potency and stability of the toxoid against RVV in other countries may not be fruitful from the cost-benefit point of view.

References

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Russell’s Viper (*Daboia russelii siamensis*) Toxoid:
Variation in Biological Properties of the Toxoid and Influence of Immunization Schedule on Antibody Response

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**Abstract**

Biological properties of formal-treated Russell’s viper (*Daboia russelii siamensis*) toxoid stored at +4°C were monitored by the WHO recommended methods at 2, 3, 4, 6, 10 and 12 month following preparation. Formal toxoiding of the venom resulted in reduction of 15-fold in lethal, 4-fold in coagulant, 22-fold in defibrinogenating, 6-fold in haemorrhagic and 10-fold in necrotic activities. A 44-fold reduction in capillary permeability increasing activity of the toxoid was observed from four months onwards. Further two-fold reduction in hemorrhagic and necrotic activities of the toxoid was observed from six months onwards. Decrease in number of precipitin bands and protein bands in SDS-PAGE electrophoresis was observed when the toxoid aged. Such variation in biological properties of stored toxoid will influence the quality of antibody produced and hence the protective immunity of the immunized subjects.

The influence of immunization schedule of Russell’s viper toxoid on antibody response in immunized rabbits and monkeys were studied. Rabbits were immunized subcutaneously with the toxoid at 0, 4 and 8 wks and subsequent boosting was carried out at 6 wks intervals from 24 to 56 wks and up to 40 wks in monkeys. Antibody levels peaked at 6-8 wks after the third injection and fell to low level at 24 wks after the first injection. Subsequent boosting at 6 wks intervals from 24 wks onwards resulted in increase in antibody level reaching peak at 12 wks after the first booster injection and sustained at its peak throughout the course of immunization. Antibody response observed in the monkeys was similar to the rabbits, however a lower level of antibody was observed. Feasibility and cost-effectiveness of boosting at 6 wks intervals in prophylactic active immunization of farmers at risk with Russell’s viper toxoid needs to be studied.

**INTRODUCTION**

Monitoring of biological properties of toxoid on storage was not included in the earlier studies on formal-treated Russell’s viper toxoid (Khan et al., 1980, Aung-Khin et al., 1980).
Reversion of formalin linkage with venom in Russell’s viper toxoid stored at 4°C and 37°C after three months has been reported (Aye-Kyaw et al., 1992). The objective of this study is to monitor the biological properties of Russell’s viper (Daboia russelii siamensis) toxoid during storage at 4°C (Tun-Pe et al., 1995a).

The pattern and development of antibody following three injections (0, 4, 8 wks) of Russell’s viper (Daboia russelii siamensis) toxoid was studied up to six months in monkeys and human volunteers (DMR working groups, 1986, a, b) and 64 weeks in rabbits (Aye-Aye-Myint et al., 1996). However, the latter study indicated that boosting with aged toxoid at variable intervals failed to induce antibody response in some instances. This extended study is to find out the influence of immunization schedule of the toxoid on antibody response in rabbits and monkeys (Aye-Aye-Myint et al, 1998).

MATERIALS AND METHODS

Toxoiding of crude Russell’s viper (D. r. siamensis) venom (batch 90) (Myanmar Pharmaceutical Factory) was carried out by stepwise formalinization method (Kondo et al., 1971, Aung-Khin et al., 1980). The final preparation containing 2 mg protein/ml and aluminium phosphate was aliquoted and stored at 4°C. Biological properties of the toxoid such as lethality (LD50), haemorrhagic (MHD), necrotic (MND), coagulant (MCD), defibrinogenating (MDD) and capillary permeability increasing activities (MCPID) were monitored by WHO recommended method (Theakston and Reid, 1983) at 2, 3, 4, 6, 10 and 12 month following preparation. Immunogenicity of the toxoid was compared with the crude venom using 1% agarose gel immunodiffusion plate (Ouchterlony, 1958). SDS-PAGE electro-phoresis of the unreduced toxoid was run on 12% separating gel (Laemmlı, 1970).

Immunization of Rabbits

Two sets of rabbit (three tests and one control) each weighing 2.5 kg were injected subcutaneously (s.c.) with 0.1ml fresh toxoid at five different sites on the back of shaved dorsal skin at 0, 4 and 8 wks. Subsequent immunizations were given at six wk intervals from 24 to 54 wks. Controls received adjuvant only. Two ml of blood were collected at 0, 4, 8 wks before giving the toxoid and at two wks intervals up to 54 wks from all rabbits. Serum sample was aliquoted and stored at -20°C (Aye-Aye-Myint et al., 1998).

Immunization of Monkeys

The same immunization and sample collection schedules were used for immunizing 12 monkeys (8 tests and 4 controls) up to 40 wks. Each was separately caged and s.c. injection was given at deltoid region. Controls received preparation containing adjuvant-phosphate buffered saline.

Local reactions such as swelling, ulceration, abscess formation, regional lymph node enlargement and general constitution symptoms such as alertness, eating habits, general well being and limitation of movement of arm were recorded six hourly for 24 hours and
daily for a week after each immunization in monkeys. For rabbit experiments, only local reactions were recorded (Aye-Aye-Myint et al., 1998).

**Biological properties of the toxoid on storage**

Biological properties of the toxoid of varying ages were shown in figure (1).

Formal toxoiding of the venom resulted in reduction of 15-fold in lethal, 4-fold in coagulant, 22-fold in defibrinogenating, 6-fold in haemorrhagic and 10-fold in necrotic activities. A 44-fold reduction in capillary permeability increasing activity of the toxoid was observed from 4 months onwards. Further two-fold reduction in heamorrhagic and necrotic activities of the toxoid was observed from six months onwards. (Tun-Pe et al., 1995b)

Quantitative decrease in number of precipitin bands and protein bands in SDS-PAGE electrophoresis was observed when the toxoid aged (Fig. 2)

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**Figure 1.** Biological properties of Russell’s viper toxoid of varying ages

**Figure 2.** Immunodiffusion (left) and SDS-PAGE electrophoresis (right) of Russell’s viper toxoid of varying ages

LD50 iv = lethality, MDD = minimum defibrinogenating dose, MCD = minimum coagulant dose, MHD = minimum haemorrhagic dose, MND = minimum necrotic dose, and MCPID = minimum capillary permeability increasing dose.

V=venom, To=freshly prepared toxoid, T8= 8 months old toxoid, M=markers and ASV=antivenom
RESULTS

**Antibody response following immunization with the toxoid**

Peak antibody level was reached in rabbits four weeks after the third injection of the toxoid and it fell to low level at 24 wks after the first injection. Further boosting at six wk intervals from 24 wks onwards resulted in peaking of antibody response four wks after the second booster dose given at 32 wks and peak antibody level was maintained through out the course of immunization i.e. upto 56 wks (Figure 3) (Aye-Aye-Myint et al., 1998).

A similar pattern of antibody response was observed in the immunized monkeys; however, the peak antibody level achieved was low compared to that obtained in the rabbits (data not shown).

**Reactions following immunization with the toxoid**

Local reactions following immunization in the rabbits were: local induration measuring 1 cm in greater diameter which lasted for 48 hours was observed in 4/6, 3/6 and 2/6 after the first, second and third injection respectively and local swelling measuring 2 cm in greater diameter was detected in 2/6 and 1/6 after the second and the third respectively. No reactions were detected in the controls.
Local reactions following immunization in monkeys were: local swelling measuring 1 cm in diameter which lasted for 48 h was observed in (3/8), ulceration (1/8) after the first injection and local swelling measuring 2 cm in diameter which lasted for 48 h was detected in (1/8), regional lymph node enlargement (1/8) and abscess formation (1/8) after the second. No reactions were observed in subsequent injections. Only one control showed an induration of 1 cm on second injection.

Upset in general constitution symptoms was not observed in immunized monkeys (Aye-Aye-Myint et al., 1998).

DISCUSSION

Formal toxoiding leads to decrease in toxicity far greater than that produced by gamma irradiation (100 Krad) of venom (3-fold LD_{50}, 1.75-fold MHD and 4.5-fold MND (Mandal et al., 1993). The MCPI activity of the venom was not affected until four months of storage. Further 44-folds decrease in the activity and two-fold reduction in haemorrhagic and necrotic activities of the aged toxoid will further weaken the neutralizing properties of the antivenom raised. The capillary permeability increasing activity is exclusively seen in Russell’s viper (D.r. siamensis) bite cases of Myanmar and its presence signifies a bad prognosis. Four to 22 folds reduction in biological activities of the toxoid at two months of storage calls for use of either freshly prepared toxoid or toxoid stored up to two months in active prophylactic immunization against Russell’s viper bite (Tun-Pe et al., 1995).

Peak antibody level observed 4-6 weeks after last injection in the present study (Aye-Aye-Myint et al., 1998) was similar to that observed in our earlier study (Aye-Aye-Myint et al., 1996) where intramuscular route of immunization was used. However antibody level is short-lived and could not be maintained upto 24 wks after the first injection of the toxoid. There was a slow induction of secondary response when booster doses were given at 24 and 30 wks. Probably intramuscular route of administration induces a quicker antibody response. Boosting at the decline of antibody level was found to induce a more rapid response (Aye-Aye-Myint et al., 1996) than that given at a time where antibody level is very low as in our present study.

The age of the toxoid has no influence on the pattern of antibody response. The interval of boosting plays an important role in induction and maintenance of peak antibody response. Frequent inoculation at weekly to two weekly intervals with venom or toxoid have been used for active immunization in man against venom which yielded high level of neutralizing antibody (Flowers, 1963, Weiner, 1960).

High resting antibody level is essential in immunized individual since immediate neutralization of injected venom is required when a snake bites a man. It could be achieved by boosting at six week intervals from eight week after the third injection of toxoid. The feasibility and cost effectiveness of frequent injections at six week intervals to the risk population needs to be studied.
References


INTRODUCTION

As in many developing tropical countries, snakebite is of great importance but in Myanmar, it enjoys the attention it deserves. The National Health Committee has pronounced snakebite the eleventh priority in the National Health Plan. It is also one of the 17 diseases under national surveillance. Snakes and snakebite have played a great part in the lives of the Myanmar people even before colonial times. Much research has been done on snakes, snakebite and their prevention and management since the mid 20th century, particularly by the Department of Medical Research, the Insein and Yangon General hospitals, among others. Snakebite morbidity and mortality continue to be an important health problem especially, because antivenom was in short supply and the management dictated by several experts confused the health personnel even further, not to mention the local snake man, some monks and others who firmly and sincerely believe that theirs is the best form of management.

The major venomous snakes of Myanmar responsible for bites are Russell’s viper (Viper Russelli) 90%, cobras (cobra Naja Naja & King cobra Naja Hannaha), krait (Bungarus faciatus), sea snake (Hydrophis Strictcollis), green snake species (Trimeresurus trimeresurus & others) 10%, Rarer species yet to be reported.

The Department of Health Planning, Ministry of Health and this department also record snakebite. Although it is not possible to record all cases of snakebite, all cases of poisonous cases bites are recorded. However, those who do not come to the health facilities, or die on the way are not recorded, as are those who are bitten by unknown snakes and do not show features of envenoming; thus the condition is underreported. However, as these recordings are made by qualified statisticians from the same are in the same way, the figures are reliable and provide the minimum figures and a working basis. Although no age or sex is exempt, two-thirds of the victims are young healthy male cultivators the age specific morbidity is between 15 and 45 years, and the majority are breadwinners of the family resulting in great socioeconomic loss. It is mainly the men who prepare the soil often working knee deep in water, but they are joined by the women for planting and harvesting the rice and pulses. 25% of the victims are women. Snakebite morbidity was more than 10 000 annually and the mortality ran in thousands despite under reporting. Out of the need to “control” the situation “The snakebite control project” came into existence in 1994 in the National Health Plan. The snakebite control project is under the Directorate of Health services, Ministry of Health. In 1996, it was included in the regular
budget of the World Health Organization. The annual budget was around US$ 25 000 and has been reduced to half this year.

**The current situation**

After an initial rise at the beginning of the snakebite component of the National Health plan (probably due to the increased awareness) there has been a gradual decline and the morbidity and mortality has been reduced to morbidity 7 000 and mortality of 500 in 2000. (Fig. 1)

The majority of those affected were healthy males in the most productive age range i.e. 15–45 years. The socio economic impact of the loss of these lives is obvious. However, no age or sex or occupation is exempt.

The areas with the highest incidence of snake bites are: Mandalay, Magwe, Sagaing, Ayeyarwaddy, Bago, Yangon divisions and the coastal regions of Myanmar and the project targets these areas.

The reason for the increased initial morbidity could be increased awareness, increased farming and development activities without effective preventive measures such as footwear and lighting while traveling to and from work. This has now been reduced by nearly 50% compared to the start of the project in 1994 (Fig. 1).

The mortality figures have not changed significantly although the morbidity has been reduced; this means that the percentage of people dying has increased. The mortalities are due to: improper identification of the snake, lack of proper first aid measures, delay in the administration of antivenom, due to patients coming to hospital only after swallowing the snakes tail and reciting mantras, lack of antivenom which does not reach the rural health centre level owing to inadequate production by the Myanmar Pharmaceutical Factory.

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**Figure 1. Poisonous snakebite, Myanmar (morbidity & mortality)**

The mortality figures have not changed significantly although the morbidity has been reduced; this means that the percentage of people dying has increased. The mortalities are due to: improper identification of the snake, lack of proper first aid measures, delay in the administration of antivenom, due to patients coming to hospital only after swallowing the snakes tail and reciting mantras, lack of antivenom which does not reach the rural health centre level owing to inadequate production by the Myanmar Pharmaceutical Factory,
Ministry of Industry 1, lack of adequate facilities at various levels to deal with the complications of snakebite.

**Antivenom**

The supply and distribution of antivenom is one of the most important factors, as if given early and adequately prevents many of the complications which are both expensive and difficult to treat. Cobra bite patients are the best examples of this because the symptoms are neurotoxic and less complicated than that of Russell’s viper bites and studies have shown that early adequate administration at the time of the onset of ptosis is associated with recovery without the need for ventilation.

Many people arrive after several hours or even days to the health facility and in these people, and very severely envenomed patients, antivenom is not the sole solution. There is a need for the proper care of the severely envenomed patient immediately. This entails extensive education of both medical and lay persons in the effective first aid measures potent, timely and adequate antivenom administration together with strengthening of the health infrastructure to support critically ill patients. Practical training of health personnel in the management of snakebite patients is the most effective as the medical officers have personal experience in the management of these patients and are then capable of making on the spot decisions correctly and confidently as to whether or not to give antivenom and decide on the dose. This will save lives as well as prevent wastage of antivenom on those who do not need it.

Storage of liquid antivenom in cool places and the use of watered clay pots (described also in this seminar) in order to cool the antivenom cannot be practised during the several days’ journey from the medical depots to the rural health facility, so that meticulous storage in clay plots will only help to maintain the potency that remains. Hence, freeze dry antivenom must be produced.

**Freeze-dried antivenom**

Increased production of this form of antivenom was initiated by the Snakebite Control Project and through the concerted effort of UNDP/WHO, a freeze dry machine and generator were purchased in 1999/2000. Help is required in this area as well as others areas involved in increase production of potent freeze-dried antivenom (see Fig. 2). The majority of poisonous snake bites are due to viper bite and the annual requirement is approximately 100 000 ampoules of 10 mg vials ie 50 000 of 20 mg vials. The current production is approximately 30 000-vials of 20 mg/annum of viper and cobra or 60 000 vials of 10mg. Less than 10% of this freeze dry. The liquid antivenom requires cool temperatures to maintain its potency and it is impossible to maintain the temperature continuously during transport and storage. Imported antivenom is not fully effective in the case of viper bite due to the subspecies variation of vipers.

The morbidity and mortality from sea snake bites affecting the fishing and marine industry cannot be ignored. As there is no antivenom available in the country for this...
Snakebite Control in Myanmar

snake, the majority of victims remain in their village and treat themselves with traditional methods like trying to keep awake. This antivenom produced from Enhydrina schistosa is effective for all sea snake bites. But further study of the situation is necessary before any recommendations can be made regarding this expensive item.

There is no antivenom produced (available) in Myanmar for the fatal King Cobra bite which occurs in the rural areas and in the forest. Antivenom from Thailand has been used and found to be effective in a case of accidental bite occurring in Yangon Zoological gardens. Small quantities of this can also be purchased and made available to foresters and in rural areas.

Green snakebite though not fatal is responsible for much morbidity producing severe local swelling and wound necrosis leading to many days loss of labor and occasionally loss of limb. Antivenom for this snake is not produced locally as Russells’ viper and cobra are priorities.

Snake eradication measures have been tried in early 20th century and this resulted in an increase in rodent population and a fall in the yield of paddy and pulses. Further there is an added risk of plague and other rodent borne diseases. Snake meat and rat meat is considered a delicacy by some and selective destruction of poisonous snakes and rodents with sparing of non poisonous snakes could reduce the incidence of poisonous snake bites without upsetting the ecology.

Figure 2. Number of vials of Antisnake venom (20 mg/vial) purchased from Myanmar Pharmaceutical Factory by Central Medical Store Depot

Immunodiagnosis for detection of venom from DMR when available will be utilized effectively in the diagnosis and management of snakebite cases. Fibrin Degradation Product kits also from DMR will also aid in the management of these patients.
Care of the severely envenomed patients

Owing to the NHP 1994-2001, new snakebite centers opened peritoneal dialysis facilities—3 in Magway Division, 2 in Sagaing division, 1 in Mawlamyine, 1 in Ayeyarwaddy and 1 in Yangon Division. Critically ill-patients are best managed with minimum disturbance. Hence, dialysis on site is best with regard to outcome not to mention the transport expenses and social inconveniences involved. Also, under the National Health Plan 1994-2001, the snakebite control project established *Haemodialysis* facilities for selected snakebite renal failure patients (hypercatabolic patients with less bleeding tendencies), in the central snakebite training center in Sanpya Hospital, Thingangyun. This is associated with shorter clinical course and duration of hospital stay (results to be published).

Training courses for basic health workers and doctors in the identification of poisonous snakes, prevention of bites and management are conducted by the snakebite control project. Life support training is being conducted by the cardiovascular disease project and is important for neurotoxic envenoming before the patient reaches the health facility.

**AIM AND OBJECTIVE OF THE SNAKEBITE CONTROL PROJECT**

To reduce the morbidity and mortality from snakebite:

**Strategies**

1. Health education. Health education on prevention, training in care of envenomed patients targeted at the lay public, personnel as appropriate using all available methods of mass education. Targeting the lay public, basic health workers, doctors and nurses, NGOs, religious groups, patients and attendants; snakebite prevention and the use of protective footwear well accepted by the lay public through public education, better information regarding identification, first aid and management throughout Myanmar especially focussing on the areas of greatest incidence, the patients themselves as well as their relatives and friends.

2. Production and distribution of health education material.

3. Practical training in management and making peritoneal dialysis available in township hospitals in endemic areas.

4. Advocacy and coordination in obtaining material for the production of more and better antivenom. Appropriate and adequate distribution of antivenom. More freeze dried antivenom. The Ministry of Industry I produces the antivenom, the dialysis fluids supplies of which are important in reducing the mortality from snakebite. Therefore, inter-ministry cooperation and coordination are essential.

Health education is focused on:

- Prevention of snake bites, administration of antivenom and intensive care management are not 100% successful. (At the same time, cautioning against killing off of snakes stressing on the snakes contribution to medicine, the environment and rodent control).
- Identification of poisonous snakes and features of envenoming, as this aids in the prompt management of the patient, reducing mortality.
- What to do when bitten by a snake. The teaching of practical methods of first aid using instantly available material and why it should be done. Focal groups such as medical personnel, NGOs religious groups are targeted, as they are the groups capable of spreading the technique in the community.
- Care of the envenomed patient: Education of medical personnel regarding the care of the envenomed patient.

This led to a fall in morbidity to less than 8 000 poisonous snake bites a year (from more than 10 000 in 1991).

- Multiplier training courses bi annually in prevention, identification, first aid and management in the case of health personnel.
- Practical training courses are conducted in the snakebite control center in Sanpya Hospital to doctors in endemic areas in the management of snakebite, assessment and treatment providing hands on experience in peritoneal dialysis and witnessing of haemo-dialysis. All divisions have been covered but not well. The courses continue. This training also improves the skills and quality of health care in other patients with renal failure.
- All available media for public education. Multimedia for mass education, radio talks once a year, Television programmes viewed on national and Myawaddy channels with nationwide coverage.
- Magazine and newspaper articles one a year or more.
- Production and distribution of health education material. This is distributed during training courses, to snakebite victims and their attendants through the ministries of Information, Transport, development of national races and border areas, education, agriculture, school health, occupational health, Central Health Education Bureau, NGOs, Maternal and Child Welfare Associations, Red Cross, Patients and attendants in the wards, video houses.

**Health Education Materials Produced and Distributed Since 1994**

1. Myanmar and the danger of poisonous snakes in Myanmar 30,000 copies
2. Snakebite control for primary health care providers English 4,000 copies
3. Posters on first aid 6,000 copies
4. Poster on prevention 4,000 copies
Management of Snakebite and Research

(5) Poster on important venomous snakes of Myanmar, 6,000 copies
(6) Flipchart on important venomous snakes of Myanmar, 4,000 copies
(7) Flipchart on “When a snake bites......” 4,000 copies
(8) Pamphlet on “The danger of poisonous snakes” 50,000 copies
(10) Advocacy and coordination in obtaining material for the production of more and better antivenom, leading to adequate distribution of antivenom

More Freeze-dried Antivenom

Coordinating the Myanmar Pharmaceutical Factory, Ministry of Industry 1, (where the antivenom is produced) and the Ministry of Health which distributes the antivenom, to the health facilities where the patients are treated.

The antivenom production is complex. It requires obtaining and keeping of healthy horses big enough for adequate bleeding. There is batch to batch variation of the potency of various fractions of antivenom. The liquid antivenom requires careful storage and instructions. Freeze dry machines and generators are required for the more stable freeze dry antivenom.

A proposal made by the project manager using methods from Australia for producing antivenom IgY from chickens to run concurrently was turned down.

The greatly needed freeze dry machine was procured by UNDP and WHO and installed in the Myanmar Pharmaceutical Factory since the year 2000 and is now being used to produce some of the freeze dry antivenom. The strength of each vial of Russell’s viper antivenom produced was converted from 20 mg neutralizing capacity to 10 mg neutralizing capacity so that in comparing production the number of vials of 10 mg neutralizing strength must be divided by two and the production over the past few years has not increased significantly.

Although there is a gradual fall in the morbidity rate, mortality rate has not fallen (Fig. 1) probably due to improper first aid measures and inappropriate management, late arrival, inadequate and delayed administration of antivenom and loss of potency of antivenom.

Benefits

• Reduction in snakebite morbidity and mortality.
• Improved health care facilities and capabilities of health personnel for critically ill patients resulting in improved health care infrastructure.
• Reduced morbidity reduces the health care burden and economic loss.

RECOMMENDATIONS

• The quality and management of antivenom supply should be enhanced for improved outcome of the victims of snakebite.

• More dialysis centres should be instituted to help in the reduction of mortality from snake bites and other acute renal failure cases such as gastroenteritis, leptospirosis, septic abortion, malaria, accidents and trauma. These critically ill patients, too ill to travel, can be dialyzed without delay.

• Intensive care centres should be set up in endemic townships.

• Immunodiagnostic kits should be made available to the medical personnel to improve the diagnostic and assessment skills and lead to a fall in mortality from snakebite.

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A Dipstick Test for Rapid Diagnosis of Russell’s Viper (*Daboia russelii siamensis*) Bite (A 20 minute Russell’s viper dipstick)

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**OBJECTIVES**

- To develop a robust rapid test for diagnosis of Russell’s viper bite
- To select and estimate antivenom doses required for treating the case

**RATIONALE**

- Russell’s viper bite is an occupational hazard of our farmers.
- Only 44% of the victims brought dead snakes for identification.
- 28% of the victims are not envenomed.
- 50% of non envenomed cases are given 1-4 ampoules of antivenom.
- At present- selection of antivenom for treating snakebite cases is based on:
  - If snake is brought -species identification
  - If no snake is brought -syndromic approach plus circumstances of the bite
- A rapid diagnostic test is required for:-
  - Identification of inflicting snake
  - Selection of an appropriate antivenom
  - Estimation of antivenom dose
  - Minimize wastage of expensive antivenom
- A rapid enzyme immunoassay dipstick test developed
  - needs to use expensive reagents
  - takes 40 minutes to get the result.
- A less expensive and more rapid diagnostic test is urgently needed.
MATERIALS AND METHODS

(1) Nitrocellulose paper 0.22µ
(2) Russell’s viper venom (RVV) (Myanmar Pharmaceutical Factory)
(3) Bovine Serum Aibunim fraction V
(4) Affinity purified rabbit anti-RVV IgG
(5) Dye antibody conjugate

Method

The principle of the test is double antibody sandwich dot blot.

Preparation of a dipstick

A nitrocellulose strip is coated with rabbit anti-RVV IgG antibody and blocked with 5% Bovine Serum Aibunim in Phosphate Buffered Saline. Then, it is incubated with venom standard (60ng/ml) and human serum (negative control) for 15 min. at room temperature and rinse with tap water and dry. It is stored in a sealed polythene bag at 4°C (six months) and room temp (three months).

Testing of a patient’s serum sample

A test strip is laid on a flat surface, add one drop of serum to the test spot and incubate for 15 min. at room temperature. Then, it is rinsed with tap water, dried and add one drop of dye antibody conjugate to test, standard and control. If it is positive, a red ring develops.

Interpretation

A red ring indicates a venom level of 10 ng/ml or more. The venom level is estimated by comparing the colour intensity of the ring with the standard (60ng/ml Russell’s viper venom). No colour develops in control.

• An insulin syringe is used for delivery of sample and dye conjugate
• Dye antibody conjugate is freshly reconstituted with 100 µl of the diluent, stored at 4°C and used within two weeks.

RESULTS

A. Sensitivity of the test –10ng/ml of RV venom

B. Specificity–does not cross react with other snake venoms.
**Performance of the test**

<table>
<thead>
<tr>
<th>Colloidal Dye Immunoassay (Dipstick Test)</th>
<th>Enzyme Immunoassay</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>22</td>
</tr>
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</table>

Specificity 100% (95% CI 81.5-100)
Sensitivity 97.0% (95% CI 88.7-99.5)
Positive predictive value 100% (95% CI 93-100)
Negative predictive value 91.7% (95% CI 71.5-98.5)
Kappa coefficient 0.9414
One tailed p value 0.0000 (<0.001)

**DISCUSSION**

- A robust dot blot dipstick for rapid diagnosis of Russell’s viper bite is developed.
- Specific and sensitive – detects a venom level of 10ng/ml (usually seen in local envenomed cases)
- Rapid- result within 20 min. compared to 40 min. of enzyme immunoassay dipstick.
- Simple- to perform and requires no instrument nor expertise.
- Economical-uses one tiny drop from an insulin syringe of dye conjugate and serum
- Inexpensive – does not need to use expensive enzyme and substrate.
- Less expensive than enzyme immunoassay dot blot dipstick.
- Costs – 450 kyats per test kit.
- Could be adapted for diagnosis of other snakes.
- A long shelf life- six months at 4°C and three months at room temp.
- Specific uses
  - Identification of snake species
  - Estimate venom level of the victim
A Dipstick Test for Rapid Diagnosis of Russell's Viper

- Selection of antivenom
- Estimate antivenom dose required.

Other usefulness

- Estimation of venom level after antivenom therapy
- Estimation of venom level of prospective turns severe envenoming cases.
- Help in early institution of antivenom in prospective turns severe envenoming cases

National benefits

- Helps in saving of giving antivenom to non-envenomed cases.
- Helps in estimating antivenom dose and reduces antivenom abuses.
- Helps in cutting national spending on antivenom.