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CRITICAL CARE

Hump-nosed pit viper (*Hypnale hypnale*) envenoming causes mild coagulopathy with incomplete clotting factor consumption

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Context. Limited information exists on the coagulopathy caused by hump-nosed pit viper (*Hypnale hypnale*) envenoming. *Objectives.* This study aimed to characterise the coagulopathy in hump-nosed pit viper bites by measuring laboratory clotting times and factor studies. *Materials and methods*. Cases of hump-nosed pit viper envenoming were included from a prospective cohort study of Sri Lankan snake-bite patients. Patient age, sex, snake identification, time of bite and clinical effects were recorded. Patients did not receive anti-venom because no specific anti-venom to hump-nosed vipers exists. All patients received supportive care and serial 20-min whole blood clotting tests (WBCT20). The prothrombin time (PT), international normalised ratio (INR), activated partial thromboplastin time (aPTT), coagulation factors I, II, V, VII, VIII, IX and X, von Willebrand factor (vWF) antigen and D-Dimer concentrations were measured. The median of highest or lowest test result for each patient was reported with interquartile range (IQR). *Results.* There were 80 hump-nosed pit viper bites, median age was 37 years (IQR: 26–51 years) and 48 were male. The WBCT20 was positive in one patient. The median highest INR was 1.9 (1.5–2.2; Range: 1.3 to > 12) and median highest aPTT was 54 s (46–72 s; Range: 35–170 s). There was low fibrinogen [median: 1.3 g/L;1, –1.8 g/L; Range: < 0.2–2.9], low factor VIII levels [median: 23%; 16–37%] and low factor V levels [median: 43%; 23–74%]. D-Dimer concentrations [median: 3.4 mg/L; 2–7.4 mg/L] were slightly elevated. Factors II, VII and X and vWF antigen concentrations were normal. *Discussion and Conclusions.* Hump-nosed pit viper bites result in a mild coagulopathy which is usually not detected by a WBCT20. It is characterised by mild elevation of INR, low fibrinogen and Factors V and VIII which may be consistent with the venom containing a thrombin-like enzyme.

Keywords Clotting factors; *Hypnale*; Hump-nosed viper; Coagulopathy; Snake envenoming; Venom

Abbreviations aPTT, activated partial thromboplastin time; INR, international normalised ratio; IQR, interquartile range; MCC_5, minimum clotting concentration defined as the venom concentration that caused clotting after 5 min; PT, prothrombin time; SV-TLE, snake venom thrombin-like enzyme; VICC, venom induced consumption coagulopathy; VWF:Ag, von Willebrand factor antigen; WBCT20–20 min whole blood clotting test

Introduction

Snake envenoming is now recognised as a major health issue in the rural tropics and in particular in south and southeastern Asia.[1] Venom-induced consumption coagulopathy (VICC) is one of the most common snake envenoming syndromes and results from pro-coagulant snake toxins activating the clotting pathway and causing deficiencies of clotting factors.[2] There is limited information on the severity of VICC and the specific factor deficiencies that occur with different snake species.[3–6] In many cases a 20-min whole blood clotting test (WBCT20) is the only test used to detect and monitor coagulopathy in snake bite. More comprehensive investigation of VICC is important for an understanding and treatment of snake envenoming.

Hump-nosed pit viper (Genus *Hypnale*) bites are considered to be the commonest cause of snake bite in Sri Lanka.[1] Currently three species are recognised in the genus *Hypnale*: *H. hypnale*, *H. nepa* and *H. zara.*[7] Bites by Merrem's hump-nosed pit viper (*H. hypnale*) are commoner than the other two species[8] due to the wide distribution of this species around Sri Lanka.[7] Previous reports and clinical experience suggest that envenoming by hump-nosed pit vipers mainly

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causes local effects and less commonly coagulopathy and acute kidney injury.[8,9]

Although coagulopathy is reported to be uncommon, it is the most common systemic effect reported in *Hypnale* envenoming.[9–13] *In vitro* studies of *Hypnale* venom show it has mild pro-coagulant activity consistent with clinical reports.[14] However, different studies report different incidences of coagulopathy, from 4%[8] to 39%.[9] Except for one study,[13] the presence of coagulopathy is based on a positive WBCT20,[9–12] which has been shown to be unreliable because there is no standardisation of the method.[15] This is the likely reason for the reports of different frequencies of coagulopathy. Coagulopathy, fibrinolysis and spontaneous systemic haemorrhage have also been described following *H. hypnale* envenoming in India.[16]

One previous study of *Hypnale* envenoming showed that 12 of 56 patients had a positive WBCT20, but no evidence of spontaneous bleeding.[13] Ten of these patients had an increased level of fibrinogen degradation products, and seven of these had reduced fibrinogen levels. None of the patients had any abnormalities in their bleeding time, platelet count, prothrombin time (PT) or partial thromboplastin time with kaolin. It is likely that the WBCT20 will only be positive for severe coagulopathy, so mild to moderate coagulant effects may have been missed in this study.

The frequency of coagulopathy and the importance of VICC in *Hypnale* species envenoming remains unclear. The aim of this study was to characterise the severity and frequency of coagulopathy in Sri Lankan hump-nosed pit viper (*H. hypnale*) bites by measuring laboratory clotting times and factor studies for definite hump-nosed pit viper bites.

Methods

This was a prospective observational study of patients with definite hump-nosed pit viper (*H. hypnale*) bites presenting to Chilaw hospital in Central West Sri Lanka. It was conducted as part of a large cohort study of snake bites presenting to the one hospital.[17] The study was approved by the Ethical Review Committee, Faculty of Medicine, University of Colombo. All patients gave written and informed consent for the collection of data and blood samples.

Patients

Cases of hump-nosed pit viper (*H. hypnale*) envenoming were included between January 2007 and July 2009. Any patient older than 13 years of age and who presented with a snake bite was identified when they arrived in hospital. Snake bite cases were initially considered for the study if the patient brought in the snake and health care staff identified it as a hump-nosed pit viper. Inclusion criteria were cases of suspected hump-nosed viper bites where Sri Lankan hump-nosed pit viper (*H. hypnale*) venom was detected in the patient's serum with venom-specific enzyme immunoassays (EIA). *H. hypnale* is the only *Hypnale* species that occurs in this Central West region of Sri Lanka. Patients taking an anti-coagulant or platelet inhibitor were excluded.

Data collection

Baseline data, including demographic features (age and sex), bite information (species of snake and time of bite), clinical effects (local effects: local pain, swelling, bruising, blistering and necrosis; systemic effects: clinical evidence of coagulopathy including bleeding, neurotoxicity and myotoxicity) complications and treatment were recorded for all patients. The WBCT20 was performed routinely on admission for all patients and repeat testing every 6 h was done by the treating team until discharge.[4,18] Research blood samples were collected from all patients on admission and then after 6 h, 12 h, and every day until discharge, if they did not receive anti-venom. Blood was collected in citrated tubes for coagulation studies and in serum tubes for venom-specific EIA. All samples were immediately centrifuged, aliquoted and frozen at -20°C and then transferred to a -80°C freezer within 2 weeks of collection until the completion of the study.

None of the hump-nosed pit viper bites received anti-venom because there is no species specific anti-venom to hump-nosed pit vipers and none of the Indian polyvalent snake anti-venoms currently used in Sri Lanka include antibodies to hump-nosed viper. All patients received supportive care. All decisions regarding treatment were made by the treating clinicians.

Venom-specific EIA

Frozen patient serum was used to test for the presence of *H. hypnale* venom using an EIA. The EIA uses polyclonal antibodies (IgG) raised in rabbits to *H. hypnale* venom. Detection uses biotinylated antibodies and then streptavidin horseradish peroxidise. The limit of detection of the assay is 0.2 ng/mL and the method has been previously described in detail.[17,19,20]

Clotting studies and clotting factor assays

Frozen patient citrated plasma was subjected to a series of clotting tests at a centralised laboratory and included PT [and international normalised ratio; INR], activated partial thromboplastin time (aPTT), levels of factors I (fibrinogen), II (prothrombin), V, VII, VIII, IX and X, von Willebrand factor (vWF) antigen and D-Dimer as previously described.[21] All assays were performed on either a Behring Coagulation System or Sysmex CA-1500 analyzer (Dade Behring, Marburg Germany) using standard coagulometric or immunoturbidometric methods as provided by the manufacturer.

Individual coagulation factor levels were determined by incubating patient plasma and factor deficient plasma with the relevant activator, and the time for clot formation was measured in seconds. For each clotting factor, the amount of factor available in the system was quantified from a standard or reference curve of known factor concentrations versus clotting time. The quantification of vWF:Ag and D-Dimer was done using immunoturbidometric methods.

Statistical analysis

The average number of samples collected per patient was three. Clotting studies and factor levels were measured on

Table 1. Lowest (or highest) value for the clotting tests or factor level results for the 80 patients during their hospital admission. Data is presented as median and 2.5–97.5 percentiles.

	Hump-nose viper	Normal range
INR[†]	1.9 (1.3–7.1)	0.9–1.3
aPTT (s)[†]	54 (37–133)	25–35
Fibrinogen (g/L)[*]	1.3 (< 0.2–2.3)	2–4
Factor II (%)	79 (49–129)	70–120
Factor V (%)	43 (5–115)	70–120
Factor VIII (%)	23 (0.9–85)	70–150
Factor VII (%)	85 (35–163)	70–120
Factor IX (%)	73 (38–125)	70–120
Factor X (%)	85 (43–155)	70–120
vWF Ag (%)	77 (23–189)	50–160
D-dimer (mg/L)	3.4 (0.2–49)	< 0.5

INR, international normalised ratio; aPTT, activated partial thromboplastin time; vWF Ag, von Willebrand factor antigen.
*The limit of detection for fibrinogen is 0.2 g/L.
[†]An INR of 12 was unrecordable, i.e. > 12 and an aPTT of 180 was unrecordable i.e. > 180 s.

all samples for each patient. The highest (INR, aPTT, vWF anti-venom and D-Dimer) or the lowest (fibrinogen II, V, VII, VIII, IX and X) of each test for all samples done in an individual patient was reported for each patient for that test. Descriptive data is presented as medians with interquartile ranges (IQR) and ranges. All analyses and graphics were done in GraphPad Prism version 5.03 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

Results

There were 80 patients with definite hump-nosed pit viper (*H. hypnale*) bites from an initial 94 patients with suspected hump-nosed pit viper bites based on patient or hospital staff identification. The median age 37 years (IQR: 26–51 years) and 48 were male. Local envenoming occurred in 74 (92%) patients. Local effects were reported as follows: local pain, 72 (90%); local swelling, 11 (14%); local blistering, 4 (5%) and local necrosis, 1 (1%). The WBCT20 was positive in one patient (1%) on admission. No patient developed clinical evidence of systemic coagulopathy (including bleeding).

The median highest INR and median highest aPTT in patients bitten by the hump-nosed pit viper were elevated at 1.9 (IQR: 1.5–2.2; Range: 1.3 to > 12) and 54 s (IQR: 46–72 s; Range: 35–170 s), respectively. These abnormal clotting times were associated with low fibrinogen levels [median, 1.3 g/L; IQR: 1.0–1.8 g/L; Range: < 0.2–2.9], low factor VIII levels [median 23%; IQR: 16–37%] and low factor V levels [median 43%; IQR: 23–74%] (Table 1 and Fig. 1). D-dimer concentrations [median 3.4 mg/L; IQR: 2–7.4) were slightly elevated. Factors II, VII and X and vWF antigen concentrations were within the normal range (Table 1). There was no difference in the coagulopathy between patients with and without local effects.

Discussion

This study has shown that hump-nosed pit viper envenoming caused a mild coagulopathy in all patients which in most cases was not detected by a WBCT20. It is characterised by a low but detectable fibrinogen, and low levels of Factors V and VIII. However, the INR was similar to that of patients on therapeutic anticoagulants and none of the patients developed any clinically detectable signs of coagulopathy.

The presence of a mild coagulopathy in all hump-nosed pit viper bites in this study is the likely explanation for the inconsistency seen in previous reports of the coagulopathy. These studies used the WBCT20 to determine the presence of coagulopathy following *Hypnale* envenoming and reported a

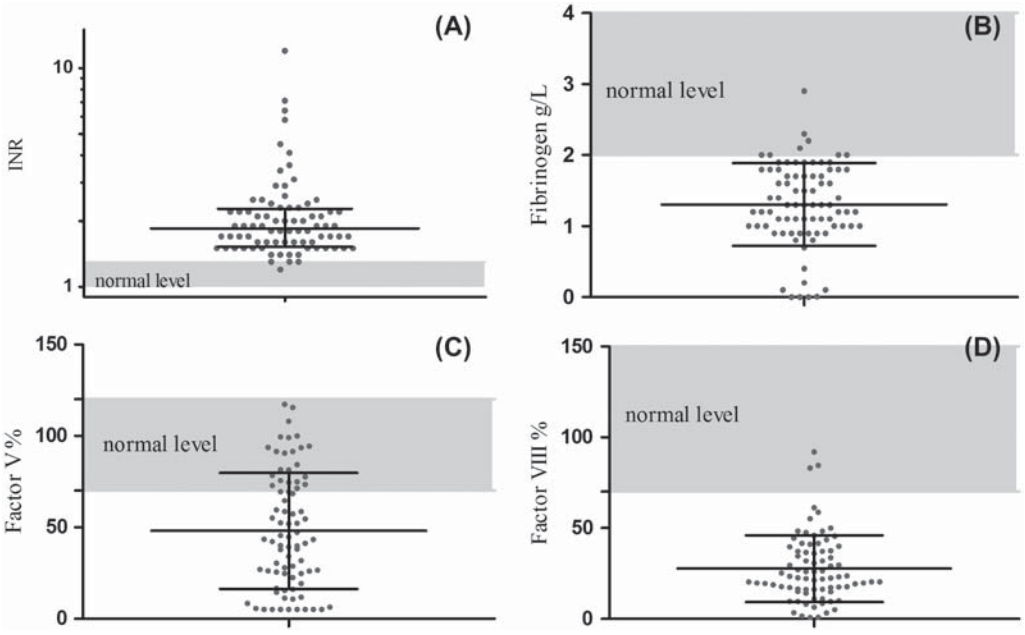

Fig. 1. Scatter plots of peak INR (A), lowest fibrinogen (B), lowest Factor V (C) and lowest Factor VIII (D) with medians and interquartile ranges in 80 patients with hump-nosed pit viper envenoming.

prolonged WBCT20 in 39% of patients in one study,[9] and more recently only 4 of 93 patients (4%).[8] These reports demonstrate the questionable reliability of the WBCT20 for detecting the mild coagulopathy following envenoming by hump-nosed pit vipers. There were six patients with evidence of coagulopathy and who had no reported local effects. This suggests that not all patients with hump-nosed viper envenoming develop significant local effects.

A number of *in vitro* studies have investigated the pro-coagulant effects of *Hypnale* venom. Thrombin-like enzyme activity of *H. hypnale* venom has been reported as 20–22 s/50 µg of venom.[22,23] A more recent study found mild pro-coagulant activity of *H. hypnale* venom with a minimum clotting concentration 5 (MCC_5) value of 4.4 µg/mL.[14] This value is 10- to 50-fold greater (i.e. lower pro-coagulant activity) than 0.08–0.4 µg/mL for Australian elapids, which have highly potent pro-coagulant venoms.[24] These studies would suggest that *Hypnale* venom has a mild thrombin-like effect most likely from the presence of a snake venom thrombin-like enzyme (SV-TLEs) in the venom.

SV-TLEs can act on either the α, β, or both chains of fibrinogen and produce corresponding fibrinopeptides. This leads to consumption of fibrinogen (defibrination) rather than conversion to fibrin and cross-linking to form a clot.[25] Such defibrination is seen commonly with the Malayan pit viper (*Callosellasma rhodostoma*) which is the phylogenetically closest pit viper to *Hypnale* and whose venom contains Ancrod which has fibrinogenolytic Aα activity.[26] It is therefore likely that the hump-nosed pit viper venom contains SV-TLEs which is consistent with the results of our study in which low fibrinogen levels were observed.

The low D-Dimer results in this study are interesting and we assume their slight elevation occurred because very little fibrinogen was converted to fibrin and cross-linked. Thus, the fibrinolysis will not result in cross-linked degradation products (XDPs), and only fibrinogen degradation products (FDPs). The latter are not detected by the newer D-Dimer assays. In contrast, Australian elapids produce VICC that results in very high D-Dimer (100- to 1000-fold elevations) because they contain prothrombin activators that result in the normal conversion of fibrinogen to fibrin with cross-linking, followed by fibrinolysis.[3]

The low concentrations of Factor VIII compared to Factor V in *Hypnale* envenoming are unexpected considering the mildness of the coagulopathy. The Factor VIII concentrations are similar to those seen with VICC following Australasian elapid envenoming, but the latter also causes complete consumption of fibrinogen and Factor V.[3] It is unlikely that this reduction of Factor VIII is just secondary to the consumption of fibrinogen in the case of *Hypnale* envenoming, because Factor V levels are only mildly depressed. Another possibility is that the major pro-coagulant toxin or perhaps another toxin in hump-nosed pit viper venom can activate Factor VIII. Some of the SV-TLEs are known to activate other factors in the clotting cascade,[2] including Factor VIII.[27] Thrombin-like activity in association with Factor VIII activation by *Hypnale* venom could be the possible explanation for the above coagulation results.

One limitation of the study was that timed coagulation tests and clotting factor studies were not available for the full duration of hump-nosed pit viper envenoming. Patients with hump-nosed viper bites were often discharged within 24 h of admission due to the mild clinical effects. Further studies will need to measure clotting tests over a longer period of time to determine when complete recovery of haemostasis occurs. Another limitation of the study was that frozen samples were used for the clotting studies and factor levels. However, this has been done previously in an Australian study.[21] This approach has the advantage of all the studies being done within a short period of time in one laboratory. All samples were centrifuged immediately after collection, aliquoted and then frozen by clinical research assistants who had been trained in sample collection and processing.

Conclusions

This study reports coagulation tests and clotting factor levels following hump-nosed pit viper envenoming showing that it causes mild VICC with mildly decreased levels of fibrinogen and Factor V, and low levels of factor VIII. These findings are consistent with at least SV-TLEs being present in *Hypnale* venom. This study demonstrates the limitations and ongoing problems with using WBCT20 to define coagulopathy in snake-bite patients.

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Ethical statement

The study was approved by the Ethical Review Committee, Faculty of Medicine, University of Colombo.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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