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Factor assay in victims of snake bite: Experience from a tertiary care institute in South India

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Abstract:

INTRODUCTION: Snake bites tend to cause a high mortality in those who develop coagulopathy. However, there is very limited literature on clotting factor assays in these patients, especially in the presence of clinical bleeding. The aim was to assess the coagulation profile and individual coagulation factors in patients with hematotoxic snake bites.

MATERIALS AND METHODS: This was a prospective observational study of clotting factor levels in victims of snake bites with hematotoxicity admitted to a single hospital in south India for 12 months. In 43 individuals who fulfilled the criteria, we measured platelet count, prothrombin time (PT), international normalized ratio, activated partial thromboplastin time (aPTT), fibrinogen levels, coagulation factors V, VII, VIII, IX, and X, and the qualitative factor XIII assay.

RESULTS: Forty-three patients fulfilled the criteria and their samples were studied. There were 36 Russell's viper (Daboia russelli), 4 Hump-nosed pit viper (Hypnale hypnale), and 3 unknown snake bite victims samples, in which factor assays were done. All the Russell viper bite victims without a recordable clotting screen had deficiency of Factor V (0.5%–49.62%, Mean – 20.27%), Factor X (0.08%–92.3%, Mean – 70.73%), and qualitative factor XIII. Pit viper patients showed normal levels of Factor I, V, VII, VIII, IX, X, and XIII despite prolonged PT and aPTT.

CONCLUSIONS: Early detection and treatment of envenomation remains the cornerstone of managing snake venom-induced consumptive coagulopathy. Anti-snake venom plays a major role in the reversal of coagulopathy. Blood and blood products would be useful when coagulopathy does not revert by ASV alone. Evidence-based transfusion can be implemented and cryoprecipitate may be used as many of the patients had factor XIII and fibrinogen deficiency as part of venom-induced coagulopathy. To improve patient management and thereby the outcome of patients CMEs and training programs for the treating physicians also has to be implemented so that guidelines are formulated and followed.

Keywords:

Coagulation, factor assays, snake bite, venom-induced coagulopathy

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Introduction

Snakebite is yet to be regarded as a disease in a country like India where we possibly have the largest number of snake bite-related death in the world.[1] The venom of various species of snakes found in Kerala, namely Daboia russelli, Hypnale hypnale, and Trimesurus malabaricus are known to cause hematotoxicity.[2] There are no studies from this part of the country that examines the *in vivo* effect of envenomation by these species on the coagulation parameters. This study intended to look at the coagulation profile of patients bitten by hematotoxic snakes.

Materials and Methods

Study design and setting

This was an observational study conducted prospectively over a 12 month period after

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approval from the Institutional Ethics Committee and Research Committee (Ref No: 41/12/IEC/JMMC& RI) in a tertiary care referral center for snake bite victims in Kerala, South India. This center caters to the need of the patients from in and around Thrissur District in Kerala.

The patients were managed as per the institutional snake bite treatment protocol which adheres to the World Health Organization[3] and national snake bite treatment protocol.[4]

Data collection

All snake bite patients were interviewed using a standardized questionnaire to maintain a record of the patient's history relevant to snake bite envenomation. Information about the victim's status, its management (first aid/traditional treatment), and the time between bite and reaching the hospital was obtained in each case. In the event the bitten snake was brought along the specimen was identified by an expert and data recorded.

Patients

All snake bite patients were admitted for observation and those who did not develop any signs and symptoms of envenomation were discharged after at least 24 h of observation. If the snake specimen brought was a hump noised pit viper the institutional protocol was to observe for a period of 48 h.

Coagulation parameters studied included platelet count, prothrombin Time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), and factor assays for factor I (fibrinogen), V, VII, VIII, IX, X and the qualitative factor XIII assay using the urea clot solubility test.

Only the cases fulfilling all the following criteria were included in the study

Inclusion criteria

1. Patient with an alleged history of snake bite
2. Patients with prolonged 20 min whole blood clotting test (20'WBCT).
3. Patients with one or more clinical manifestations of snake bite like local swelling, active hemorrhages including but not limited to gum bleeds and ongoing ooze or bleed from the bite site, vomiting, abdominal pain, or newly tender regional lymphadenopathy.

Patients with any of the following were excluded:

Exclusion criteria

1. Patients with no signs of envenomation during the period of observation
2. Patients not consenting to be part of the study
3. Patients getting discharged against medical advice or those not willing for admission and observation even though warranted
4. The snake specimen brought and identified as non-venomous
5. Patient with a history of bleeding disorder
6. Chronic alcoholics
7. Patients on anticoagulation therapy
8. Snake bites in patients <12 years of age.

Coagulation studies

All patients fulfilling the criteria were assessed using a 20 min WBCT (20'WBCT). The 20'WBCT was repeated every 30 min up to 3 h, then every 6 h for 24 h. If the bite is known to be from a hump-nosed pit viper, the 20'WBCT was repeated six hourly for an extended period of 48 h, even if the initial evaluation by 20'WBCT was normal. Victims who had a prolonged 20'WBCT were considered to be envenomed and were included in the study after getting consent from the nearest relatives.

The cohort of investigations included; a complete blood count, bleeding time, PT, INR, and aPTT. A citrated blood sample was collected at the time of the first deranged 20'WBCT from which the plasma is separated and frozen at minus 80° for coagulation factor assays. All Blood tests and assays were done in the institution itself using the ACL top 300 analyzers (Werfen/Instrumentation Laboratory).

Data analysis

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements were presented on mean ± standard deviation (Min-Max) and results on categorical measurements were presented in Number (%). Significance was assessed at a 5% level of significance.

The following assumptions on data were made, Assumptions: (1) Dependent variables should be normally distributed, (2) Samples drawn from the population should be random, cases of the samples should be independent.

Chi-square/Fisher's exact test was used to find the significance of study parameters on categorical scale between two or more groups.

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Significant figures

- Suggestive significance (P value: $0.05 < P < 0.10$)
- Moderately significant (P value: $0.01 < P < 0.5$).

** Strongly significant (P value: $P \leq 0.01$).

Statistical software

The Statistical software, namely SAS 9.2, IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp , Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver. 2.11.1 were used for the analysis of the data. Microsoft word and Excel have been used to generate Graphs and Tables.

Results

There was no one who took treatment from traditional healers before coming to our hospital compared to the other parts of the country where more than half of the victims seek treatment from traditional healers before they present to the hospital.

The maximum incidence of snake bites was in the rainy season. About 75% of our snake bite cases occurred during June. Males comprised 63.8% of the snake bite patients [Table 1]. Twenty-five percent of the patients reached the hospital within 60 min and received treatment. Seventy percent of the patients reached the hospital within one to 2 h time.

A total of 445 patients presented to the emergency department with an alleged history of snakebite. A total of 163 patients were given anti-snake venom (ASV) due to some signs of envenomation. They were admitted to the Medical ICU for further observation and treatment. Only 43 patients fulfilled the criteria and their samples were separated and collected for the study. There were 36 Russell's viper (Daboia russelli), 4 Hump-nosed pit viper (Hypnale hypnale), and 3 unknown snake bite patient samples, in which factor assays were done [Figure 1].

Myalgia and pain at the bite site were seen in all the patients. Abdominal pain and vomiting were present in 83% of our patients who showed envenomation.

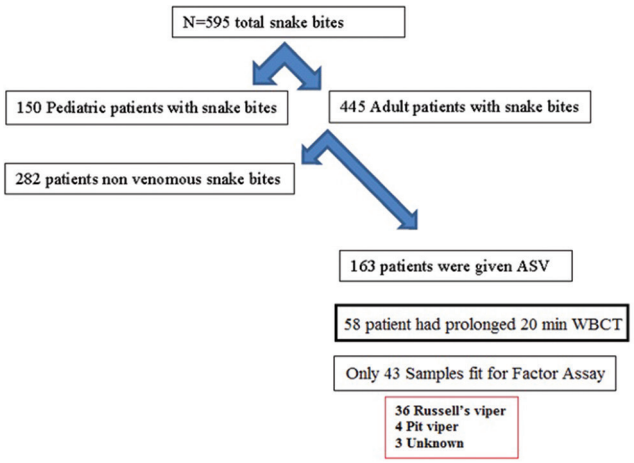

Figure 1: Study population

Forty percent of the patients had bleeding from the site of bite, which is considered a very common bleeding manifestation. Other manifestations include generalized ecchymosis, purpura or hematomas, frank or microscopic hematuria, hemoptysis, gingival bleeding, hematemesis, melena, and in severe cases patients went into shock. Arterial thrombosis has been described as a local complication of bites by some vipers, but none of our patients developed arterial thrombosis [Table 2].

All these 43 samples had prolonged PT and APTT [Table 3]. The coagulation factor assays for factors 1, V, VII, VIII, IX, X, and qualitative factor XIII were done on these samples. All the Russell viper bite victims without a recordable clotting screen had deficiency of Factor V (0.5%–49.62%, Mean – 20.27%), Factor X (0.08%–92.3%, Mean – 70.73%) [Table 4], and qualitative factor XIII [Table 5]. This finding substantiates that the venom in Russell's viper is Factor X and V activators. The high amount of Factor VIII (8.66%–472%, Mean–100.69%) was also seen in a patient. In our study, 30 patients post-Russell's viper bite had qualitative Factor XIII

Table 1: Age distribution

Age in years	Gender		Total
	Male	Female	
11-20	11(10.6%)	3(5.1%)	14(8.6%)
21-30	18(17.3%)	5(8.5%)	23(14.1%)
31-40	14(13.5%)	14(23.7%)	28(17.2%)
41-50	27(26%)	17(28.8%)	44(27%)
51-60	16(15.4%)	12(20.3%)	28(17.2%)
61-70	14(13.5%)	8(13.6%)	22(13.5%)
>70	4(3.8%)	0(0%)	4(2.5%)
Total	104(100%)	59(100%)	163(100%)

Table 2: Clinical manifestations in various snakes

Clinical features	Snake					Total (n=163)
	Cobra (n=4)	Krait (n=2)	PIT (n=9)	RV (n=66)	UK (n=82)	
Abdominal pain	0(0%)	1(50%)	9(100%)	56(84.8%)	68(82.9%)	134(82.2%)
Vomiting	2(50%)	1(50%)	9(100%)	56(84.8%)	68(82.9%)	136(83.4%)
Myalgia	4(100%)	2(100%)	9(100%)	62(93.9%)	80(97.6%)	157(96.3%)
Pain of bite site	4(100%)	2(100%)	9(100%)	66(100%)	82(100%)	163(100%)
Blurring Vision	4(100%)	2(100%)	0(0%)	0(0%)	0(0%)	6(3.7%)
Localized edema	4(100%)	0(0%)	9(100%)	66(100%)	82(100%)	161(98.8%)

Pain at bite site was seen in all snake bite patients
Abdominal pain, vomiting and Myalgia are seen in 85% of the bite victims and can be considered as a very early sign of envenomation

Blurring of vision was seen only in neurotoxic snake bites
Localized edema was not seen in any of the krait bites

Table 3: Activated partial thromboplastin time in comparison with prothrombin time

Activated Partial Thromboplastin Time	Prolonged time		Total
	No	Yes	
Normal	40(34.5%)	8(17%)	48(29.4%)
Not tested	74(63.8%)	19(40.4%)	93(57.1%)
Prolonged	2(1.7%)	20(42.6%)	22(13.5%)
Total	116(100%)	47(100%)	163(100%)

P<0.001**, significant, Chi square test

20 patients had both PT & APTT prolonged

Table 4: Coagulation screen and factor assays

Recordable Coagulation screen of six Russell viper patients

Parameters	Normal	Mean	Minimum	Maximum
PT (in sec)	10 TO 13	23.01	14	48.6
INR	1-1.3	2.17	1.32	4.58
APTT (in sec)	30-34	77.76	30.6	222.9
Fibrinogen (mg/dl)	150 -250	179.9	100.89	299.07

Factor Assays of the patients with recordable coagulation screen

Factor	Normal (%)	Mean (%)	Minimum (%)	Maximum (%)
V	50 TO 150	20.27	0.57	49.62
VII		97.52	37.12	233.177
VIII		45.58	25.56	101.17
IX		126.9	15.35	245.23
X		70.73	0.08	92.3

Table 5: Qualitative factor XIII

Factor 13 (Using 5M Urea)	Snake		
	Pit	Russell's Viper	
		Recordable PT	Unrecordable PT
Clot present	4	6	0
Clot soluble	0	0	30
Total	4	6	30

deficiency which was diagnosed by urea clot solubility test.

In our study, 3 out of the 4 pit viper bite case had a prolonged 20′ min WBCT even after 24 h without any evidence of major bleeding manifestation. The coagulation screen showed deranged PT/INR, APTT, and Fibrinogen. Factor assays were done on these patients, which showed normal amounts of all coagulation factors tested. None of the Pit viper patients had a qualitative Factor XIII deficiency.

Discussion

Clinical syndromes of snake bite are relatively species-specific, but only a half of the victims in our study were able to accurately identify the species of the snake. A better public awareness about early attendance at a specialized center is required to reduce the complication and mortality rate. This is evident in the fact that all the patients that reached our center within 30 min of the bite were discharged earlier and had no clinically significant morbidity.

The focus of the study was on coagulopathy and its clinical manifestation of bleeding. The incidence of bite-site bleeding was similar to another study by Bhat and Reid *et al*. The study done by Bhat *et al*. was a prospective cross-sectional study conducted on 100 patients, for analysis of clinical features with the evaluation of coagulation disorder in patients presenting with symptoms, signs and definite evidence of snake bite. All patients were studied at the time of admission, before and after administration of ASV and were treated and followed up in the hospital until recovery or death.

Often, signs of systemic poisoning are not always clinically evident and hence, any evidence of bleeding would be an indication for administering ASV. A delay in this respect can often cause more of the venom to act on the clotting cascade and worsen the coagulation defect. We observed that the period between the snake bite and the onset of coagulation disorder varied from person to person between one to several hours depending on the amount of venom injected and the species of the snake. Bhat *et al*.[3] reported that only 3% of his patients developed bleeding within the first 6 h after the bite while the majority of the patients (83%) developed hemorrhages between 7 and 48 h and 13% developed hemorrhages even after 48 h.

In our study, we had seven patients who had normal first 20 min WBCT but a prolonged second WBCT, mainly those who were pit viper bites.[3] This observation would suggest all patients with viper bites should be kept under close observation for at least 48 h since a delayed development of coagulation defect may be overlooked and can result in life-threatening bleeding.

The coagulation factor assay done on these patients showed a deficiency of Factor V and Factor X with varying amounts of other factors. This finding substantiates that the venom in Russell's viper is Factor X and V activators. A consumptive coagulopathy results from the activation of the clotting pathway by procoagulant toxins in the venom. The snake venom components that act on the coagulation system are classified according to the part of the coagulation pathway where they act and include factor V activators, factor X activators, prothrombin activators, and thrombin-like enzymes or fibrinogenases. The severity, duration, and type of

consumption coagulopathy differ depending on the type of procoagulant toxin.[4,5]

The high amount of Factor VIII was seen in a case. This has also been observed by other researchers, especially in the early phase of envenomation.[6] A possible explanation for this finding is that the presence of "Russell's viper venom factor X activator toxin" in the sample results in falsely high factor levels. The *in vitro* activity of the toxin would appear similar to the activity of factor VIII. It may also be due to the endothelial activation, leading to the release of more Factor VIII.[7,8]

Lalloo *et al*.[9] reported 13 cases of factor XIII deficiency in patients who developed coagulopathy following snake bites. In our study, 30 patients after Russell's viper bite had qualitative Factor XIII deficiency by the urea clot solubility test. None of the Pit viper patients had a qualitative Factor XIII deficiency. Factor XIII assay was not done. Point-of-care testing like TEG/Rotem would be helpful in detecting coagulopathy in similar situations.

Few of the limitations of this study are that most of the snake bites were due to unknown or unidentified snakes. Samples of all the bite patients could not be analyzed fully, either due to unavailability of the sample or early death of the patient or improper collection, leading to hemolysis of the sample.

Conclusions

To improve the patient management and thereby the outcome of snake bite patients; continuing medical education and training programs for the treating physicians also have to be implemented so that guidelines are formulated and followed.

The authors would like to recommend the following to improve patient outcome. Evidence-based transfusion can be implemented. Cryoprecipitate can be used as many of the patients had factor XIII and fibrinogen deficiency after snake bite.

Guidelines have to be formulated and followed. There is an important requirement for a protocol for laboratory investigations and hemotherapy in snake bite patients which has to be formulated in the institution.

As prevention is always better than cure, public awareness, and population-based programs have to be conducted to spread the message of snake bite prevention, first aid, and early treatment which are the main pillars of adequate snake bite management.

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Conflicts of interest

There are no conflicts of interest.

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