Original Research Article

A study on clinical and laboratory features of pit viper envenomation from Central Kerala, India

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ABSTRACT

Background: Snakebite envenomation is an important public health problem faced by the tropical countries with India, the worst affected in terms of mortality and morbidity. In spite of increasing reports of other snake species causing envenomation, the existing research and management strategies including antivenom are still focused on the “Big Four” species- Russel’s viper, saw scaled viper, common krait and spectacled cobra. Pit vipers as a group are being increasingly reported to cause human bites from different parts of the country. Hence, we decided to study the clinic-epidemiology of pit viper bites.

Methods: 30 cases of proven pit viper bites who attended our Department during the study period of 18 months were analysed for the epidemiological factors, clinical features and abnormalities in laboratory parameters.

Results: Hump nosed pit viper (Hypnale hypnale) was responsible for all the thirty cases. 57% of cases were females. Mean age of victims was 41.8 years. 17 patients had exclusively local envenomation. Ten cases had coagulopathy along with local envenomation. Three patients developed acute kidney injury of whom three underwent dialysis. No mortality was observed in the study. Low fibrinogen levels were observed in all cases with coagulopathy and some had low levels of factor V (70%) and factor VIII (40%).

Conclusions: Hump nosed pit viper bites were observed to be common in this part of the country. Significant envenomation can occur. Further epidemiologic studies involving more centres will be helpful in quantifying the true incidence of bites. Since no specific antivenom is available, further researches in this direction are warranted.

Keywords: Envenomation, Hypnale hypnale, Pit viper

INTRODUCTION

Snakebites and their associated mortality continue to be an important public health problem in many tropical and subtropical countries.1 All the available statistics related to snakebite envenomations implicate South Asia to be the world’s most affected region by snakebites. Country wise India ranks first in terms of incidence as well as mortality associated with snakebites.2

Though there are more than 60 venomous snake species in our country, traditionally only four- Naja naja (common cobra), Bungarus caeruleus (common krait), Echis carinatus (saw-scaled viper), and Daboia russelii (Russell’s viper) are considered to be responsible for the majority of envenomations.3,5 However it is increasingly being recognized that apart from these, many additional species can cause potentially lethal envenomations.6

Pit Vipers belong to Viperidae family but differs from true vipers. More than 20 species of pit vipers are found in India and neighbouring countries.4 Hump nosed pit vipers (Hypnale hypnale) are reported mainly from Srilanka.7,8 Recently reports from Kerala also have pointed towards clinically significant envenomation from this species.9,10 Similarly Malabar Pit Viper
(Trimeresurus malabaricus) also has been implicated in envenomations from Karnataka. In the wake of these reports it was felt a need to study the clinical manifestations and abnormalities in laboratory parameters along with mortality figures (if any) in proven pit viper bites.

**METHODS**

The study was a Hospital-based observational study carried out for 18 months (April 2014 to September 2015).

**Inclusion criteria**

- A proven case of pit viper bite
- Age: 15 years and above

A proven case is defined as those bite cases in which the snake has been brought and has been identified as pit viper by the investigator.

**Exclusion criteria**

Any pre-existing disease known to cause coagulopathy/bleeding according to history.

30 cases of pit viper bites admitted over the study period were enrolled.

The study population was selected from the cases of snake bite presented to the hospital. We analysed only those patients who have brought the concerned snake. If the species is identified as any of the pit viper species then the patient was enrolled in the study after getting a written and informed consent.

**Methodology**

Data was collected employing a proforma. All the basic sociodemographic details were collected with special focus on the geographic location and the setting in which the bite occurred- timing, locality etc. An initial clinical assessment was carried out to look for any evidence of local or systemic envenomation.

Patients were also monitored for the development or worsening of features of envenomation every day. Special attention was given to progression of local reactions, daily urine output and obvious bleeding manifestations.

All the basic investigations were sent at admission including preliminary coagulation tests bleeding time (BT) and clotting time (20min WBCT).

Baseline values of prothrombin time (PT) and activated partial thromboplastin time (aPTT) were also obtained for every patient. Bleeding time (BT) and clotting time (20min WBCT) was done at 6th hourly intervals during the initial 24 hours and repeated everyday thereafter. If the screening tests are found to be abnormal at any time during the hospital stay then specific tests were carried out to identify the pathology causing derangement in these parameters. If bleeding time was prolonged then we did platelet count measurements. Similarly if CT was prolonged we tested the prothrombin time (PT) and activated partial thromboplastin time (aPTT). In these group of patients we kept the plasma separated and frozen. The preserved samples were subsequently used to carry out detailed coagulation studies including coagulation factor assays and mixing studies.

**Mixing studies using pooled normal plasma**

This technique was done to infer the presence of any clotting factor inhibitor. The plasma sample with a deranged coagulation profile (PT/aPTT) is mixed with PNP (pooled normal plasma) which contains all the clotting factors.

**Principle**

In the presence of inhibitor the deranged parameters fail to normalize. On the other hand if it was a clotting factor deficiency addition of normal plasma will correct the deranged parameters.

Urine output and serum creatinine measurements were monitored daily to detect any ongoing or evolving renal dysfunction. In patients with a renal dysfunction the daily urine output and the number of dialysis done were recorded.

Local reactions were categorized depending on the severity. The number of patients requiring surgical management for the local envenomation were also recorded.

Details regarding the treatment modalities employed for the management of these cases were analysed and recorded. Outcome measures including mortality if any were also looked for.

**Statistical analysis**

The data obtained were entered into Microsoft Excel after coding for the variables. All analysis was carried out using the statistical software SPSS (Version 20). Mean was used as the measure of central tendency and standard deviation was used as measure of dispersion for descriptive statistics.

**RESULTS**

Out of 634 cases of snakebites reported to the hospital, pit vipers were responsible in 33 cases, of which 30 cases were included applying the inclusion criteria (Figure 1).
The mean age of the cases was 41.87±16.03 years. The majority (73.3%) belonged to the age group 25-65 years. Females dominated with 57% against 43% males. The majority of victims were housewives and farmers. All the patients in this study were bitten by Hypnale hypnale or the hump nosed pit viper (HNV) (Figure 2).

Most of the bites occurred in the evening between 4pm and 8pm. The boundaries however extended to early morning bites at 4.00 am and nocturnal bites at 12.00 am. The monthly distribution showed a maximum of 5 cases in October 2014 and 4 cases each in December 2015 and August 2015. Bites were relatively infrequent during January to June 2015. All the cases were reported from rural locations and all the bites were sustained from outdoor locations. The location of the bites included the vicinity of houses (43.3%), plantations (20%), rubber estates, and roads (13.3% each) and forests (10%). Almost 73% patients had applied tourniquet as a first-aid measure. Only 2 out of 30 patients sought traditional remedies before arriving hospital.

Lower limbs were bitten more than upper limbs. A definite bite mark was observed in 83.3% of cases. Transient bleeding from the bite site was observed in 66.7% of cases while others did not have any bleeding at all. The most common clinical outcome of HNV bites in this study is local envenomation with/without systemic manifestation (Table 1).

Two cases did not have any local reaction. Five patients had severe local reaction with gangrenous changes (Figure 3).

Involvement of the axillary or inguinal nodes were seen in 36.7% patients. Nausea and vomiting were seen in 4 cases whereas abdominal pain was observed in 2 cases. No bleeding manifestations were observed.

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**Table 1: Local reactions among pit viper bite patients.**

| Type of local reaction | No: of cases | Percentage |
|------------------------|--------------|------------|
| Nil                    | 2            | 6.67       |
| Oedema, tenderness or warmth not crossing joint | 8 | 26.67 |
| Oedema, tenderness or warmth crossing one joint | 14 | 46.67 |
| Bleb formation without gangrene | 1 | 3.33 |
| Gangrene with or without any of the above features | 5 | 16.67 |
| Total | 30 | 100 |

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**Figure 1: Flow-chart showing outline of the study.**

**Figure 2: Hump-nosed pit viper showing the pit organ (red arrow).**

**Figure 3: A bleb with gangrenous changes following pit viper bite.**

**Figure 4: Pattern of coagulopathy.**
Bleeding time remained normal in all subjects throughout the hospital stay. Two subjects with a prolonged clotting time showed normalization within 48 hours. For other subjects, the clotting time remained prolonged for many days. One subject had a prolonged PT and APTT on admission. Pattern of coagulopathy shown in Figure 4.

Out of 30 cases, 10 had deranged lab parameters of coagulation at some point during hospital stay though none showed any clinical evidence of bleeding. On direct mixing with pooled normal plasma, the deranged coagulation parameters got corrected in all cases. The mean delay in onset of coagulopathy was 20.9±23.3 hours after the bite. The mean duration of coagulopathy was 5.4±2.95 days.

Fibrinogen levels were below normal in all patients with coagulopathy, ranging from 36.4 mg% to 183.3 mg%. Factor V deficiency was the commonest (70%) followed by Factor VIII (40%) deficiency. The mean values of Factor V and VIII among these patients were 36.61±22.32% and 55.19±19.09% respectively.

With regard to renal function three patients met the criteria for acute kidney injury (AKI). Two of them were having oliguric AKI and had to be put on dialytic support. The patient with non-oliguric AKI had a normalized serum creatinine after 4 days of the bite. The duration of AKI in the other two patients lasted for 23 and 14 days before they attained normal renal parameters.

More than half of our patients received corticosteroids either oral, parenteral or parenteral followed by an oral tapering course. Among those with coagulopathy all except two patients were given FFP transfusion.

Local envenomation was seen in 93.3% cases. Exclusively local reaction without any systemic manifestations was observed in 56.7% cases. No mortality was noted in the study.

Factors related to outcome measures

Factors related to local reaction

The mean age of patients with local reaction was 42.39±16.07 years which was slightly higher than those without local reaction. The delay in reaching healthcare facility was marginally higher in those with local reaction. The mean total count among cases with local reaction was 9426.79±2895 which was higher than those without local reaction. The platelet count was slightly lower in those with a local reaction. These four parameters when assessed by independent t test but statistically did not show significance as predictors of local reaction.

Chi-square test was used to check if there was significant association between variables like gender, diabetes and hypertension with severity of local reaction. It was found that female gender had statistically significant association with severity of local envenomation (χ²=13.066, p=0.011). Diabetes (χ²= 0.879, p=0.928) and hypertension (χ²= 1.320, p=0.858) did not show any association with local reaction.

Factors related to coagulopathy

Independent t test was done to check for the association between age, delay in reaching hospital, TLC at admission and platelet count with coagulopathy but were not statistically significant.

Chi square test revealed that females were 2.3 times more prone to develop coagulopathy after HNV bite (OR=2.33, 95% CI: 0.466-11.693).

Factors related to AKI

Independent t test was done to check for the association between age, delay in reaching hospital, TLC at admission and platelet count with AKI. The mean age of cases was higher among those with AKI (47.33±16.78 years) compared to cases without AKI (41.26±16.16 years) though this observation was not statistically significant (t=0.597, p=0.601). Similarly, the mean delay in reaching hospital was higher in those who developed AKI (160.00±160.390 min) than who did not develop (68.70 ± 28.74 min) which again did not attain statistical significance (t=0.984, p=0.428). There was however a statistically significant association between a high TC and developing AKI (t= -2.599, p=0.015). The platelet count did not show much difference between those with or without AKI.

The association between gender, diabetic status and hypertension with AKI was analysed using Chi-square test. 7.7% of male patients and 11.8% of females developed AKI. Similarly, One out of two diabetics and one out of three hypertensives developed AKI. However, none of these observations had statistical significance. According to risk estimates hypertensives were 6.25 times more prone for ARF after HNV bites than people without hypertension (OR=6.25, 95% CI: 0.380-102.76) whereas diabetics are 13 times at higher risk of AKI (OR=13, 95% CI: 0.572-295.206).

Factors predicting duration of hospital stay

Linear regression was done to assess the relation between duration of hospital stay and quantitative variables including age, delay in reaching hospital and TLC.

Age of the patient did not have statistically significant effect on the duration of hospital stay (B=0.075, p=0.594).

The delay in reaching hospital however showed statistically significant correlation with the duration of hospital stay on linear regression analysis (B=0.151,
The presence of other species in Kerala like Trimeresurus macrolepis (Large-scaled Pit Viper) and Trimeresurus malabaricus (Malabar Pit Viper).

Seasonal variation in the incidence of bites by pit viper was also noted in this study. Cases were more in the 2nd half of the year after June. This is a common trend observed in most of the studies of snake bite epidemiology regardless of the species concerned.12,14 Cases were comparatively few in the months of January to March. However previous studies on HNV bites did not examine the seasonal preponderance of bites.

The delay in reaching hospital ranged from as short as 30 minutes to as long as 345 minutes with a mean of 78 minutes. However majority of our patients reached within 2 hours of sustaining the bite. Similar observation was also made by Suchitra et al.14 A long delay in one of our patient was because of going to a traditional treatment centre initially and spending about 4 hours there. Data from Vellore showed that only 76% of snake bite cases were hospitalized within 24 hours.18

First aid measures were taken by most of the victims. Only 3 patients didn’t resort to any first aid. Tourniquet was tied by 27 patients (90%). Among them 5 had tried cutting the bite site with a sharp object like razor blade.

A definite bite mark was observed in 25 out of 30 cases. In HNV bites Maduwaeg et al documented bite marks in all 93 cases.15 The visibility of bite marks may be masked in some cases where the local reaction is severe with gangrenous changes and if the bite site has been cut with sharp objects.

A transient bleeding from the bite site was relatively common, observed in 67% of cases in this study. In all cases bleeding was short lasting that subsided within 2 hours without any specific interventions. Local bleeding lasting for more than 10 minutes was reported in 5 out of 93 cases (5.3%) by Maduwaeg et al.19 Premawardena reported local oozing in 21.4% of HNV bites.20

Local reactions were almost universal in pit viper bites. Two patients did not have any local features. Majority had swelling, warmth and tenderness which in about half of the patients crossed one joint proximal to the site of bite. Five cases had severe gangrenous changes which required surgical treatment in most of them. In present study 17 cases (57%) had exclusively local reaction without systemic features. Another 11 cases (37%) had local reaction along with systemic envenomation. Hence the total prevalence of local reactions in our study was very high about 94%. Thus, it is observed that local envenomation which may lead to tissue necrosis is the most common clinical outcome of envenomation by this species. The predictors of local envenomation in these cases is further analysed below. This observation is consistent with other studies on HNV bites which also have shown that local reactions are the most common

The mean age of victims in this study was 41.87 years. The mean age of the study population from across the country ranged from 32 to 44 years.12,14,17,18 Two Srilankan studies focusing on pit viper epidemiology have recorded a median age of 37 and 39 years.19,20

The increased incidence of HNV bite among housewives noticed in our study can be explained by the propensity of this species to dwell near human settlements as was observed by Maduwaeg et al.21

All the 30 victims in this study were bitten by hump nosed pit vipers (HNV). No other species were seen in this part of Kerala though there are zoological data reporting

Similarly, the total leucocyte count also showed significant correlation with duration of hospital stay on linear regression analysis (B=0.002, p=0.037). It implies that a higher leucocyte counts directly correlates with a prolonged hospital course in HNV envenomation.

Chi-square test was done for exploring any association between gender, diabetic and hypertension status with that of duration of hospital stay. Female gender was associated with a longer duration of hospital stay ($\chi^2 = 7.941, p = 0.047$). Both diabetic status and hypertension however did not show any significant association with a prolonged hospital stay.

DISCUSSION

A total of 634 snakebites were admitted in the hospital during the study period. Pit vipers were responsible for 33 out of 128 cases of bites in which a venomous snake was identified thus contributing to 25.8%. Hence it is evident that pit vipers are relatively abundant in this part of India. Other recent studies have also reported pit vipers from Kerala causing envenomation. Srilankan studies have documented Hypnale species to be the most common cause of envenomation in that country. The incidence rate observed in this study should provoke further research to firmly establish the public health importance of pit viper envenomation in India particularly in the south.

In this study, majority of pit viper cases were females (57%). This was unlike most of the other studies which reported higher proportion of males.12,13 Data from Kottayam, Kerala also showed a male preponderance of 58% among 586 snake bite victims.14 One plausible explanation for a female predominance in this study is that a larger number of people were bitten from near their houses which makes females at slightly higher risk. It has been proven from Indian and Srilankan studies that hump nosed vipers are seen in proximity to human settlements and lower altitudes than other pit viper species.15,16

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morbidity associated with envenomation. Ariaratnam et al and Maduwage et al reported local reactions in 91% and 86% respectively. Premawardena reported local envenomation with swelling in all cases of HNV. There are many reports of cases with severe local reaction going for amputation and skin grafting following HNV bites.

In spite of local reaction, regional lymphadenopathy was not seen in most of the cases in our study. It was noted in 36.7% of cases. The reported incidence of lymphadenopathy in HNV bites by Maduwage et al was 10.8% which is lesser than that obtained in this study. Sellahewa reported 24.2% of cases having lymphadenopathy among 62 cases. The same author, from a larger study of 1543 cases showed 26.8% of lymphadenopathy. The most common symptoms observed with HNV bites was nausea and vomiting in 4 cases while abdominal pain in 2 cases. None of our patients had fever or headache which was however reported in a study from Sri Lanka.

Only one patient had thrombocytopenia on admission. Platelet counts were to be repeated only in those cases where there was a prolongation of BT values which did not occur in any of the cases at any time during the hospital stay. However, one patient had prolonged clotting time at admission itself. She reported to the hospital only 6 hours after the bite. The number of cases with prolonged CT increased at subsequent measurements. Hence there were 4, 5, 7 and 9 cases with prolonged CT at 6, 12, 18 and 24 hours after the bite respectively. However, 2 cases had normalization of their CT at 48 hours without any treatment. One case developed prolongation of CT at 84 hours after the bite. The patient who had prolongation of CT at admission showed prolongation of both PT and APTT at admission itself. None of the others had deranged PT or APTT at admission. Overall 10 patients developed coagulopathy at some point during hospital stay. The delay in the development of coagulopathy ranged from as early as 6 hours to as late as 84 hours. The mean delay was 20.9±23.3hours after the bite. 80% of cases who developed coagulopathy did so within 6 and 24 hours after the bite. The most common pattern of coagulopathy was prolongation of both PT and APTT. But one case each had a prolonged PT alone and a prolonged CT alone. The prevalence and pattern of coagulopathy seen in this study is consistent with the findings of Ariaratnam et al who found 39% coagulopathy in301 cases of HNV bites. Premawardena et al also found prolongation of CT in 21% cases but contrary to our findings PT and APTT values were normal in those cases. They postulated excessive fibrinolysis to be the pathologic mechanism for coagulopathy as further evidenced by low fibrinogen levels.

With regard to renal function, two patients had decreased urine output from admission onwards. An elevated serum creatinine was observed in 2 patients on admission and in 3 patients at 24 and 48 hours after the bite. One patient had a maintained urine output though her creatinine values remained elevated. She was a known diabetic as well as hypertensive. The incidence of AKI in this study thus stands at 10%. The duration of AKI in these patients ranged from 4 days to 23 days. The two oliguric patients were subsequently taken up for hemodialysis. The third patient showed a spontaneous resolution of her renal dysfunction as evidenced by her falling creatinine levels after 4 days. With dialytic support both of these cases showed a gradual resolution of AKI with steadily increasing urine output and a fall in serum creatinine. The two patients had 18 and 9 cycles of hemodialysis. The duration of renal dysfunction was 23 and 14 days in these two cases. The prevalence of AKI in this study was similar to that observed by Ariaratnam et al from Sri Lanka.

Of the 11 cases with systemic envenomation 8 had only coagulopathy, 2 had both coagulopathy and AKI while one patient had only AKI. Hence the incidence of coagulopathy in HNV as per this study is 30% and that of AKI is 10%.

Coagulation studies

Initial mixing study using pooled normal plasma corrected the deranged coagulation profile in all the 10 cases with coagulopathy. This screening method is used to infer the presence of any coagulation inhibitors. Subsequently factor assays were done to look for percentage activities of factors V, VII, VIII, IX and X. It was found that 70% of the cases had a lower than normal levels of factor V whereas 40% of them had lower levels of factor VIII. One patient who had an isolated prolongation of apt had lower factor VIII levels with normal levels of factor V. His coagulopathy reversed without any specific treatment. The levels correspond to mild deficiency of factors which probably explains the lack of clinically obvious bleeding manifestations in these cases. Similarly decrease in factor V activity probably explains the prolongation of both PT and APTT. Similarly, fibrinogen levels were found to be decreased in all the cases with coagulopathy. These findings correlate with the observations of Maduwage et al who observed low levels of factors V and VIII with an elevated INR as well as low fibrinogen in HNV cases with coagulopathy. However their observation that the coagulopathy is not usually detectable by 20min-WBCT is contradictory to the observations of our study.

There was no mortality in this study. The duration of hospital stay varied depending on the severity of envenomation. 33.3% of cases were discharged only after 1 week. Three of them had more than 2 weeks of hospital stay.

Table 2 depicts the comparison with other studies of Hijaz nosed pit viper bites.
Limitation of the study is the small sample size. Strength of the study is that it is a well conducted study. Pit viper bites were confirmed by identifying the snake and the laboratory parameters to establish coagulopathy also included.

CONCLUSION

To conclude we noticed that hump nosed viper bites are more common than expected in this part of the country. The most common clinical consequence observed was local envenomation which had instigated significant morbidity. Though it may result in significant envenomation, at present ASV is not available to treat these patients. Hence future studies needed in developing ASV effective against hump nosed pit viper.

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Table 2: Comparison with other studies of Hump nosed pit viper bites.

| Author                                      | This study          | Wijewatha et al 1990-2008 | Sellahawa June-December 1993 | Premawardhana et al August 1995-July 1996 | Ariaratnam et al Not known | Maduwage et al July 2008-2010 |
|---------------------------------------------|---------------------|---------------------------|------------------------------|------------------------------------------|---------------------------|-------------------------------|
| Period of study                             | April 2014-September 2015 |
| Number of cases                             | 30                  | 1543                       | 62                           | 56                                       | 301                       | 114 (HNV 93)                 |
| Local reaction                              | 94%                 | 100%                       | 100%                         | 100%                                     | 91%                       | 86%                          |
| Lymphadenopathy                             | 37%                 | 26.8%                      | 24.2%                        | Not mentioned                            | Not mentioned             | 10.8%                        |
| Coagulopathy                                | 33.3%               | 3.8%                       | 0%                           | 21.4%                                    | 39%                       | 4.3%                         |
| AKI                                         | 10%                 | 0%                         | 0%                           | 0%                                       | 10%                       | 1%                           |
| Other features                              | None                | Ophthalmoplegia, coma, shock (rare) | None                        | None                                     | Spontaneous systemic bleeding (18%) | Fever (10.7%), headache (14%) |
| Mortality                                   | 0%                  | 0.1%                       | 0%                           | 0%                                       | 1.7%                      | 0%                           |
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