ORIGINAL ARTICLE

# Snake Venom-specific Phospholipase A2: A Diagnostic Marker for the Management of Snakebite Cases

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## Abstract

**Background:** Snakebites are a common cause of morbidity and mortality, especially in tropical countries. Snakebites in any community are managed based on the clinical features and intravenous administration of antisnake venom (ASV). The administration of ASV is either deficient or given in excess based on clinical decisions and whole blood clotting test results. The present study is designed to analyze the level of snake venom component in the blood of snakebite in association with the clinical features.

**Patients and methods:** Blood samples were collected from the patients admitted to Karnataka Institute of Medical (KIMS) hospital with a history of snakebite considering the inclusion criteria. Serum was collected from the blood of snakebite patients before and after ASV and used to assess the level of venom-specific phospholipase A2 (PLA2) enzyme using the enzyme-linked immunosorbent assay (ELISA) method.

**Results:** Quantitative ELISA results revealed that the snake venom-specific PLA2 in the victim's blood was in the range of 0.3–1.27 mg/mL before the administration of ASV. However, the concentration of PLA2 after 24 hours of ASV administration was decreased in most of the patients. Further, it was observed that envenomation complications were directly proportional to the amount of snake venom-specific PLA2 found in the blood of the snakebite patient.

**Conclusion:** The study concludes that snake venom-specific PLA2 in the blood of snakebite patients could be used as a reliable venom marker, which helps in determination of appropriate ASV dosage in snakebite patients.

**Keywords:** Antivenom, Enzyme, Snakebite, Venom, Venom-specific phospholipase A2.

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## Highlights

In the present study, the clinical feature of the envenomed patient is directly proportional to the level of venom-specific PLA2. Venom-specific PLA2 in victims' blood serves as a potential biomarker in designing the dose of ASV based on the level of venom PLA2.

## Introduction

Snakebite remains as one of the major public health concerns particularly in rural communities living in the Indian subcontinent. Worldwide around 5 million people suffer from snakebite annually, causing 13,8000 deaths and nearly 40,0000 other permanent disabilities.[1] South Asia has been recognized as a biodiversity hotspot for venomous snake species, and the risk of snake–human encounters in this region is commonly reported.[2] There are no proper evidences indicating the precise number of snakebite cases since most of the snakebites are managed by traditional healers and are habitually not registered in the hospitals. India remains on the highest number of snakebite patients and deaths per year.[3,4] The World Health Organization identified snakebites as one of the neglected tropical diseases in 2017, suggesting a high priority for research in this part of the globe.[5] Therefore, in 2018, the World Health Assembly has cautioned the concerned member states and passed a resolution to tackle the snakebite problems in the South Asian region.[6,7]

The occupational profile like agriculture and other forest-dwelling tribes are at high risk to snakebite and more than 95% of mortality in India occur in the rural population.[8] This is due to the lack of information regarding venomous snakes and ASV. However, a high death count cannot be ascribed to lack of awareness because several victims die even after seeking medical treatment. One of the reasons could be due to lack of experience in handling and usually such cases are rarely examined or managed with ASV.[9] Studies in the Indian subcontinent among doctors and health-care professionals have considerable gaps in the ability to recognize systemic envenom and administer antivenom.[6,10,11] The reason being that snakebite management has not given much importance in the medical curriculum.[12] Therefore, health workers in the rural areas are also reluctant to handle snakebite cases because of apprehension about managing antivenom-linked adverse reaction.[13] It has been reported that 80% of the snakebite cases develop antivenom-associated adverse effects ranging from mild

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skin allergic reactions to severe anaphylaxis, pyrogenic reaction, and serum sickness.[14,15] Preclinical efficacy, pharmacokinetics, clinical symptom, immunological assay, and safety of current antivenoms are yet to be determined.[15–17] Also, optimal dose of antivenom required to administration is still debatable.[18] Overall, inadequate edification regarding management of snakebite cases is the main reason for the increased prevalence of the mortality and morbidity.

The current management approach for snakebite cases is based on the clinical features, where administration of appropriate dose of ASV for particular cases is challenging. Antisnake venom given most of the time is either deficient or given in excess based on clinical symptoms and whole blood clotting time results. Generally, snake venom contains components that are mainly produced to kill or paralyze the prey, which also helps in defensive mechanisms against predators. Snake venom mode of action depends on multiple factors such as type of enzyme present in it, snake species, age of the snake, and severity of the snakebite. In this context, PLA2 protein is the common venom component present in most of the snake species found in the Indian sub-continent. Till date, no information available regarding relation between envenomation complications and level of snake venom-specific PLA2 present in the blood of snakebite patients. Therefore, this study is carried out to assess the levels of snake venom-specific PLA2 in the blood of snakebite patients, before and after administration of ASV and its correlation with the clinical symptoms of the envenomed patients.

## Patients and Methods

### Sample Source

The study was carried out at Multi-Disciplinary Research Unit, KIMS, Hubballi, India. Blood samples were collected from the patients admitted to KIMS hospital with a history of snakebite considering the inclusion criteria.

Inclusion criteria of the present study include patients above 18 years old, willing to give informed consent and showing positive for whole blood clotting test (5 mL of fresh blood was collected by venipuncture in a clean dry glass tube and allowed to stand for 20 min. Blood that fails to clot is considered as positive). Further evidence of local reactions such as swelling, cellulitis, gangrene, blebs, bleeding at the site of bite, or any evidence of systemic envenomation features are also considered.

### Sample Collection

After recording basic demographics information and clinical symptoms, 5 mL of blood was collected before ASV administration and sent to the research laboratory (Multi-Disciplinary Research Unit). The blood sample received at the laboratory was allowed to clot at room temperature, then centrifuge at 450 × *g* for 10 min, and serum was collected in a fresh tube and stored at −20°C for further use. The collection of blood samples was repeated after 24 hours of ASV administration. All the patients received 10 vails of ASV manufactured by Biological E. Limited, Hyderabad, Telangana, India. The administered ASV was a combo pack containing lyophilized polyvalent, enzyme-refined equine F (ab') immunoglobulin. Each milliliters of ASV contain ≥0.60 mg antiserum for cobra venom, ≥0.45 mg antiserum for common krait venom, ≥0.60 mg antiserum for Russel's viper venom, ≥0.45 mg antiserum for Saw scaled viper venom, and ≤0.25 % w/v of phenol IP.

### ELISA Assay

Serum was collected from the blood of snakebite patients before and after ASV and used to assess the level of venom-specific PLA2 enzyme using the ELISA method (Sincere Biotech). The approximate time taken for ELISA assay was 1 hour and 30 minutes. Each well [Becton, Dickinson and Company (BD) Falcon™] was coated with 40 µL of soluble proteins from the blood to the precoated plate containing purified PLA2 antibodies. Further, secondary antibody tagged with horseradish peroxidase (HRP) conjugate was added to make antibody–antigen–enzyme–antibody complex. After incubation and washing plate, 3,3',5,5'-tetramethylbenzidine substrate was added to catalyze HRP enzyme. The blue color developed after enzyme substrate reaction was read at 450 nm on a microtiter plate reader (iMark microplate absorbance reader, Biorad), and absolute quantification of the unknown samples was extrapolated with the standards and expressed in mg/mL.

### Statistical Analysis

The results of PLA2 protein marker are presented as mean ± SD. The data were statistically analyzed by Analysis of Variance (ANOVA) using the software Prism version 9.

## Results

Snakebites are one of the major causes of death worldwide since decades. The management of snakebite completely relies on clinical manifestations of the patient, which are subjective. In this study, 20 snakebite patients admitted to KIMS, Hubballi during the study period (January 2020 to December 2020) were considered, out of which 16 patients were male and 4 patients were female, and the age range was 18–65 years old. Out of 20 snakebite patients, 17 patients were from agricultural occupations. Most (12 patients) of the snakebites were evidenced on the lower limb in the foot region and occurred post evening than compared to day. The snakebite victims were admitted to the hospital and administered with ASV within 3 hours of snakebite. However, for seven patients ASV is administration after 3–8 hours of snakebite. All patients considered in the study belong to Dharwad district of Karnataka state, and demographics information of all the patients are presented in Table 1.

### Envenomation Complications

Clinical symptoms vary considerably among the snakebite patients. Therefore, in this study, total six common clinical manifestations namely pulmonary, cardiovascular, local wound, gastrointestinal, hematological, and central nervous system symptoms were observed in all the patients at two different time points namely before ASV administration and after 24 hours of ASV administration (Table 2). In the present study, 20 patients were evaluated, out of which 4 patients recorded for pulmonary symptoms before ASV administration. Among these four patients, one patient recovered from pulmonary symptoms after 24 hours of ASV administration (Table 2, patient 2). Similarly, three patients improved with minimal pulmonary symptoms such as dyspnea, minimal chest tightness, mild/vague discomfort, and respiration of 20–25 bpm (Table 2, Patient no. 5, 8, and 20). Similarly, the reduced level of snake venom-specific PLA2 in the blood of these four patients after 24 hours of ASV administration signifies the direct corelation of pulmonary

**Table 1:** Demographics information of snakebite patients

| *Patient no* | *Sex* | *Age (yr)* | *Occupation* | *Site of bite* |
|---|---|---|---|---|
| 1 | Male | 44 | Farmer | Left foot |
| 2 | Male | 19 | Student | Right foot |
| 3 | Male | 39 | Farmer | Left hand |
| 4 | Male | 64 | Farmer | Left foot |
| 5 | Male | 25 | Farmer | Left hand middle finger |
| 6 | Female | 53 | Farmer | Right hand little finger |
| 7 | Male | 18 | Student | Left leg |
| 8 | Female | 38 | Farmer | Left lateral leg |
| 9 | Male | 48 | Farmer | Right foot |
| 10 | Male | 24 | Farmer | Dorsal of right wrist |
| 11 | Male | 63 | Farmer | Right index finger |
| 12 | Male | 36 | Farmer | Left foot dorsal |
| 13 | Male | 64 | Farmer | Right thumb |
| 14 | Female | 64 | Farmer | Right foot |
| 15 | Male | 24 | Labor | Left foot |
| 16 | Male | 41 | Famer | Right hand |
| 17 | Female | 36 | Housewife | Right hand thumb |
| 18 | Male | 64 | Farmer | Right toe |
| 19 | Male | 41 | Farmer | Right foot |
| 20 | Male | 37 | Farmer | Right feet |

symptoms with the level of snake venom-specific PLA2 in snakebite patients.

Further, cardiovascular symptoms were observed in nine patients before ASV administration, whereas these patients improved after 24 hours of ASV administration. On the contrary, the levels of snake venom-specific PLA2 in these nine patients were also declined, suggesting that the cardiovascular symptoms are directly proportional to the estimated levels of snake venom-specific PLA2. Moreover, local wound symptoms were observed in 17 patients before ASV administration but 1 patient experienced pain, swelling, or ecchymosed with 5–7.5 cm of bite site after 24 hours of ASV administration (Table 2, patient no. 3). However, one patient reported increased severity of local wound (Table 2, patient no. 1), while three patients were reported reduced severity of local wound symptoms after 24 hours of ASV administration (Table 2, patient no. 5, 9, and 16). Likewise, six patients presented to have no symptoms after 24 hours of ASV administration (Table 2, patient no. 2, 4, 7, 12, 15, and 19), but seven patients reported the same severity of local wound symptoms even after 24 hours of ASV administration (Table 2, patient no. 6, 10, 11, 13, 14, 17, and 18). The discrepancy in the severity of local wound observed in the present study clearly indicates that the snake venom PLA2 is not responsible in the manifestation of local wound during snakebite. Correspondingly, one patient (Table 2, patient no. 18) reported gastrointestinal symptoms before ASV administration. However, the patient was known to be recovered from gastrointestinal symptoms when observed after 24 hours of ASV administration. Likewise, snake venom PLA2 was also found to be reduced in the blood of this patient after 24 hours of ASV administration.

Further, 18 patients showed variable hematological symptoms before ASV administration out of which 13 patients showed no symptoms after 24 hours of ASV administration (Table 2, patient no. 1, 2, 3, 4, 6, 7, 9, 10, 11, 15, 17, 18, and 19). Whereas, four patients showed reduced hematological symptoms (Table 2, patient no. 5, 12, 13, and 14), but one patient reported the same severity of hematological symptoms (Table 2, patient no. 16). In the present study, 18 patients noted with hematological symptoms out of which 16 patients were directly proportional to the level of snake venom-specific PLA2 found in the blood of respective patients. Whereas, two patients showed increased level of snake venom PLA2 despite of decreased hematological symptoms after ASV administration. Additionally, four patients were recorded with central nervous system symptoms, out of which two patients showed minimal apprehension with mild headache and weakness or dizziness after 24 hours of ASV administration (Table 2, patient no. 2 and 8), and no central nervous system symptoms were observed among other two patients after 24 hours of ASV administration (Table 2, patient no. 5 and 17). Likewise estimated level of snake venom-specific PLA2 was directly proportional to the central nervous system symptoms in three patients but in spite of improvement, increased level of snake venom PLA2 was observed in the blood of one patient after 24 hours of ASV administration.

### Estimation of Snake Venom Components in the Blood

In the present study, snake venom-specific PLA2 was quantified by the ELISA method, which revealed that PLA2 was in the range of 0.43–1.41 mg/mL before the administration of ASV. However, the concentration of snake venom-specific PLA2 after 24 hours of ASV administration was estimated to be in the range of 0.29–1.16 mg/mL. The results indicated that 16 of 20 patients showed a significant reduction in snake venom-specific PLA2 after 24 hours of ASV administration. Further, two patients showed a nonsignificant reduction in the level of snake venom-specific PLA2 after 24 hours of ASV administration. On the contrary, one patient showed significantly increased snake venom-specific PLA2 after 24 hours of ASV administration, and one patient showed a nonsignificant increase in snake venom-specific PLA2 after 24 hours of ASV administration (Table 2).

## Discussion

We are aware that the clinical manifestations are the result of biochemical changes in the body due to venom components discharged during snakebites. Interestingly, it is also important to know that venom component is highly variable with extrinsic factors like geographical area,[19,20] season,[21] and diet.[22] Therefore, demographic data in snakebite study are of immense importance, which provides adequate information of snake species commonly found in the habitat, and it also helps to understand the risk factors of snakebite envenomation in the study locality. Similarly, demographic information is also valuable in the development of necessary health facilities for better management of snakebite cases. In the current study, the incidence of snakebite was found to be more among the male population and the majority of the snakebite patients belongs to agricultural families coming from rural areas. Further, it was noted that the lower limb was the most common snakebite site. Similar findings were observed in the previously reported studies, which support the present findings.[23–26] The envenomation complications are a complex interplay involving various factors such as size of the snake, geographical region, snake species, severity of snakebite, site of the bite, and the amount of venom components discharged during each bite. In the present

**Table 2:** Estimation of snake venom PLA2 and envenomation complications before and after ASV administration

| *Patient no* | *Envenomation complications* | *Before ASV* | *24 hr after ASV* |
|---|---|---|---|
| 1 | PLA2 | 1.09 ± 0.04 | 0.64 ± 0.09*** |
| | Cardiovascular system | HR: 100–125 bpm, palpitations, generalized weakness, benign dysrhythmia, or hypotension | No signs/symptoms |
| | Local wound | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site | Pain, swelling, or ecchymosis involving less than half the extremity (7.5–50 cm from bite site) |
| | Hematologic symptoms | Coagulation parameters abnormal: PT <20–25 sec, PTT <50–75 seconds, platelets: 50–100 K/mL, fibrinogen: 50–100 μg/mL | No signs/symptoms |
| 2 | PLA2 | 0.88 ± 0.10 | 0.67 ± 0.17** |
| | Pulmonary system | Moderate respiratory distress, 26–40 bpm | No signs/symptoms |
| | Cardiovascular system | HR: 100–125 bpm, palpitations, generalized weakness, benign dysrhythmia, or hypotension | No signs/symptoms |
| | Local wound | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site | No signs/symptoms |
| | Hematologic symptoms | Coagulation parameters slightly abnormal: PT <20 sec, PTT <50 seconds, platelets: 100–150 K/mL, fibrinogen: 100–150 μg/mL | No signs/symptoms |
| | Central nervous system | Severe confusion, lethargy, seizures, coma, psychosis, or generalized fasciculation | Minimal apprehension, headache, weakness, dizziness, chills, or paresthesia |
| 3 | PLA2 | 0.95 ± 0.03 | 0.57 ± 0.12*** |
| | Local wound | No signs/symptoms | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site |
| | Hematologic symptoms | Coagulation parameters slightly abnormal: PT <20 sec, PTT <50 seconds, platelets: 100–150 K/mL, fibrinogen: 100–150 μg/mL | No signs/symptoms |
| 4 | PLA2 | 0.86 ± 0.07 | 0.74 ± 0.13 |
| | Local wound | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site | No signs/symptoms |
| | Hematologic symptoms | Coagulation parameters slightly abnormal: PT <20 sec, PTT <50 sec, platelets: 100–150 K/mL, fibrinogen: 100–150 μg/mL | No signs/symptoms |
| 5 | PLA2 | 1.08 ± 0.05 | 0.40 ± 0.3*** |
| | Pulmonary system | Moderate respiratory distress, 26–40 bpm | Dyspnea, minimal chest tightness, mild/vague discomfort, and respirations of 20–25 bpm |
| | Cardiovascular system | HR: 100–125 bpm, palpitations, generalized weakness, benign dysrhythmia, or hypotension | No signs/symptoms |
| | Local wound | Pain, swelling, or ecchymosis involving less than half the extremity (7.5–50 cm from bite site) | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site |
| | Hematologic symptoms | Coagulation parameters markedly abnormal, with serious bleeding or the threat of spontaneous bleeding; unmeasurable PT or PTT, platelets <20 K/mL, undetectable fibrinogen, severe abnormalities of other lab values also fall into this category | Coagulation parameters abnormal: PT <20–25 sec, PTT <50–75 sec, platelets: 50–100 K/mL, or fibrinogen: 50–100 μg/mL |
| | Central nervous system | Minimal apprehension, headache, weakness, dizziness, chills, or paresthesia | No signs/symptoms |
| 6 | PLA2 | 1.10 ± 0.16 | 0.67 ± 0.10*** |
| | Local wound | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site |
| | Hematologic symptoms | Coagulation parameters abnormal: PT <20–25 sec, PTT <50–75 sec, platelets: 50–100 K/mL, or fibrinogen: 50–100 μg/mL | No signs/symptoms |

| | | | |
|---|---|---|---|
| 7 | PLA2 | 0.92 ± 0.05 | 0.36 ± 0.08*** |
| | Cardiovascular system | HR: 100–125 bpm, palpitations, generalized weakness, benign dysrhythmia, or hypotension | No signs/symptoms |
| | Local wound | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site | No signs/symptoms |
| | Hematologic symptoms | Coagulation parameters slightly abnormal: PT<20 secs, PTT <50 secs, platelets 100–150 K/mL, or fibrinogen: 100–150μg/mL | No signs/symptoms |
| 8 | PLA2 | 0.85 ± 0.08 | 0.42 ± 0.29*** |
| | Pulmonary system | Moderate respiratory distress, 26–40 bpm | Dyspnea, minimal chest tightness, mild/vague discomfort, respirations of 20–25 bpm |
| | Cardiovascular system | HR: 100–125 BPM, palpitations, generalized weakness, benign dysrhythmia, or hypotension | No signs/symptoms |
| | Central nervous system | Moderate apprehension, headache, weakness, dizziness, chills, paresthesia, confusion, or fasciculation in area of bite site | Minimal apprehension, headache, weakness, dizziness, chills, or paresthesia |
| 9 | PLA2 | 0.58 ± 0.22 | 0.29 ± 0.06*** |
| | Local wound | Pain, swelling, or ecchymosis involving less than half the extremity (7.5–50 cm from bite site) | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site |
| | Hematologic symptoms | Coagulation parameters abnormal: PT <20–25 sec, PTT <50–75 sec, platelets: 50–100 K/mL, or fibrinogen: 50–100 μg/mL | No signs/symptoms |
| 10 | PLA2 | 1.05 ± 0.04 | 0.56 ± 0.05*** |
| | Local wound | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site |
| | Hematologic symptoms | Coagulation parameters slightly abnormal: PT <20 sec, PTT <50 sec, platelets: 100–150 K/mL, or fibrinogen: 100–150 μg/mL | No signs/symptoms |
| 11 | PLA2 | 0.85 ± 0.08 | 0.63 ± 0.10*** |
| | Local wound | Pain, swelling, or ecchymosis involving less than half the extremity (7.5–50 cm from bite site) | Pain, swelling, or ecchymosis involving less than half the extremity (7.5–50 cm from bite site) |
| | Hematologic symptoms | Coagulation parameters abnormal: PT <20–25 sec, PTT <50–75 sec, platelets: 50–100 K/mL, or fibrinogen: 50–100 μg/mL | No signs/symptoms |
| 12 | PLA2 | 0.78 ± 0.06 | 0.44 ± 0.28*** |
| | Cardiovascular system | HR: 100–125 bpm, palpitations, generalized weakness, benign dysrhythmia, or hypotension | No signs/symptoms |
| | Local wound | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site | No signs/symptoms |
| | Hematologic symptoms | Coagulation parameters abnormal: PT <20–25 sec, PTT <50–75 sec, platelets: 50–100 K/mL, or fibrinogen: 50–100 μg/mL | Coagulation parameters slightly abnormal: PT<20 sec, PTT <50 sec, platelets: 100–150 K/mL, or fibrinogen: 100–150 μg/mL |
| 13 | PLA2 | 1.16 ± 0.09 | 0.67 ± 0.05*** |
| | Local wound | Pain, swelling, or ecchymosis involving less than half the extremity (7.5–50 cm from bite site) | Pain, swelling, or ecchymosis involving less than half the extremity (7.5–50 cm from bite site) |
| | Hematologic symptoms | Coagulation parameters markedly abnormal, with serious bleeding or the threat of spontaneous bleeding; unmeasurable PT/PTT, platelets <20 K/mL, undetectable fibrinogen, and severe abnormalities of other lab values also fall into this category | Coagulation parameters abnormal: PT <20–25 sec, PTT <50–75 sec, platelets: 50–100 K/mL, or fibrinogen: 50–100 μg/mL |

*(Contd....)*

**Table 2:** *(Contd....)*

| *Patient no* | *Envenomation complications* | *Before ASV* | *24 hr after ASV* |
|---|---|---|---|
| 14 | PLA2 | 0.46 ± 0.17 | 1.16 ± 0.10*** |
| | Local wound | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site |
| | Hematologic symptoms | Coagulation parameters abnormal: PT <50–100 sec, PTT <75–100 sec, platelets: 20–50 K/mL, or fibrinogen: <50 µg/mL | Coagulation parameters slightly abnormal: PT <20 sec, PTT <50 sec, platelets: 100–150 K/mL, fibrinogen: 100–150 µg/mL |
| 15 | PLA2 | 0.56 ± 0.12 | 0.37 ± 0.04** |
| | Local wound | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site | No signs/symptoms |
| | Hematologic symptoms | Coagulation parameters slightly abnormal: PT <20 sec, PTT <50 sec, platelets: 100–150 K/mL, fibrinogen: 100–150 µg/mL | No signs/symptoms |
| 16 | PLA2 | 0.77 ± 0.03 | 0.47 ± 0.17*** |
| | Cardiovascular system | HR: 100–125 bpm, palpitations, generalized weakness, benign dysrhythmia, or hypotension | No signs/symptoms |
| | Local wound | Pain, swelling, or ecchymosis involving half to all of extremity (50–100 cm from bite site) | Pain, swelling, or ecchymosis involving less than half the extremity (7.5–50 cm from bite site) |
| | Hematologic symptoms | Coagulation parameters abnormal: PT <50–100 sec, PTT <75–100 sec, platelets: 20–50 K/mL, or fibrinogen <50 µg/mL | Coagulation parameters abnormal: PT <50–100 sec, PTT <75–100 sec, platelets: 20–50 K/mL, or fibrinogen <50 µg/mL |
| 17 | PLA2 | 0.43 ± 0.13 | 0.67 ± 0.06 |
| | Local wound | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site | Pain, swelling, or ecchymosis within 5–7.5 cm of bite site |
| | Hematologic symptoms | Coagulation parameters slightly abnormal: PT <20 sec, PTT <50 sec, platelets: 100–150 K/mL, fibrinogen: 100–150 µg/mL | No signs/symptoms |
| | Central nervous system | Minimal apprehension, headache, weakness, dizziness, chills, or paresthesia | No signs/symptoms |
| 18 | PLA2 | 0.65 ± 0.18 | 0.58 ± 0.025 |
| | Cardiovascular system | HR: 100–125 bpm, palpitations, generalized weakness, benign dysrhythmia, or hypotension | No signs/symptoms |
| | Local wound | Pain, swelling, or ecchymosis involving less than half the extremity (7.5–50 cm from bite site) | Pain, swelling, or ecchymosis involving less than half the extremity (7.5–50 cm from bite site) |
| | Gastrointestinal system | Pain, tenesmus, or nausea | No signs/symptoms |
| | Hematologic symptoms | Coagulation parameters slightly abnormal: PT <20 sec, PTT <50 sec, platelets: 100–150 K/mL, fibrinogen: 100–150 µg/mL | No signs/symptoms |
| 19 | PLA2 | 0.77 ± 0.11 | 0.39 ± 0.07*** |
| | Local wound | Pain, swelling, or ecchymosis within 5-7.5 cm of bite site | No signs/symptoms |
| | Hematologic symptoms | Coagulation parameters slightly abnormal: PT <20 sec, PTT <50 sec, platelets: 100–150 K/mL, fibrinogen: 100–150 µg/mL | No signs/symptoms |
| 20 | PLA2 | 1.41 ± 0.13 | 0.54 ± 0.23*** |
| | Pulmonary system | Moderate respiratory distress, 26–40 bpm | Dyspnea, minimal chest tightness, mild/vague discomfort, and respirations of 20–25 bpm |
| | Cardiovascular system | HR: 100–125 bpm, palpitations, generalized weakness, benign dysrhythmia, or hypotension | No signs/symptoms |

**Significance $p < 0.01$, ***Significance $p < 0.001$, HR, heart rate; PLA2, phospholipase A2; PT, prothrombin time; PTT, partial thromboplastin time

study, variable symptoms of snakebite envenomation were recorded on the admission of snakebite patients. Local wound and hematological symptoms were predominantly common among victims followed by cardiovascular system, central nervous system, pulmonary symptom, and only one patient showed abnormalities in gastrointestinal system. Indeed, the severity of clinical symptoms variably reduced among all the snakebite patients when observed after 24 hours of ASV administration. The snakebite envenomations observed in the present study are in corroboration with that of recent studies.[27,28] Similarly, studies conducted at Maharashtra also indicated that local wound symptoms were predominantly reported in snakebite patients.[24] The variable symptoms observed in this study might be due to different snake species responsible for envenomations. However, some symptoms are known to be common for most of the Indian snake species.[29,30]

Currently, intravenous administration of ASV is the only therapy accepted for the treatment of snakebite envenomations.[31] Generally, ASV includes specific antibodies that bind or neutralize the venom proteins causing the release of venom from the receptor site.[32] Analysis of snake venom component in patient's blood is important for the estimation of dose of antivenom. However, due to the lack of appropriate assay for the measurement of venom in the patient blood, the administration of ASV is carried out based on the degree of envenomation, administration of insufficient ASV doses reduce the neutralizing potency, which contributes to an incidence of prolonged envenomation reactions. However, excess doses of ASV administration would result in adverse effects. Therefore, the selection of appropriate doses of ASV is not only crucial for the management of snakebite patients but also avoids the wastage of valuable and scarce stocks of ASV.

Generally, four venomous snake species belong to two families namely Elapidae and Viperidae are widespread on the Indian mainlands, which are also known as the "big four." They include cobra *(Naja naja)*, krait *(Bungarus caeruleus)*, saw-scaled viper *(Echis carinatus)*, and Russell's viper *(Daboia russelii)*. These specific species are responsible for the majority of snake envenomation cases in India. Since PLA2 is present in both Elapidae and Viperidae snake species,[33] in the present study estimation of snake venom-specific PLA2 was carried out in the blood of snakebite patients. Out of 20 patients, 18 patients showed decreased levels of snake venom-specific PLA2 after 24 hours of ASV administration. Correspondingly, envenomation complications were also shown to be reduced in these patients after 24 hours of ASV administration. Similarly, previous findings also uphold the results of the present study.[27] However, two patients showed increased levels of snake venom-specific PLA2 after 24 hours of ASV administration, whereas envenomation complications were found to be moderately reduced. The probable reason for increased levels of snake venom-specific PLA2 might be due to the recurrence of venom components after 24 hours, which is commonly reported in snakebite cases.[34] Another reason may be due to the administration of insufficient ASV, which fails to neutralize the high concentration of venom component in the blood of snakebite patients.[35] Further present guidelines recommend the second dose of ASV if free flowing venom component is detected after 6 hours of initial standard dose (https://nhm.gov.in/images/pdf/guidelines/nrhm-guidelines/stg/Snakebite_Full.pdf). Till date, whole blood clotting test is performed after 6 hours of initial standard dose to know the existence of free flowing venom component. However, whole blood clotting test will not sense the exact measure of free flowing venom component. Therefore, the outcome of present study suggests that snake venom-specific PLA2 could be used as a potential marker for detection of free flowing venom component.

## Conclusion

The results of the present study revealed that envenomation complications are directly associated with the levels of snake venom-specific PLA2 found in the blood of the snakebite patients. Despite the decrease in envenomation symptoms and clinically improvement of the patients, the venom component was still observed after ASV administration. Hence, the study suggests the post ASV venom measurement is crucial in the determination of unbound or recurrent venom components in the blood of snakebite patients. Snake venom-specific PLA2 assay carried out in this study provides a proof of concept that estimation of venom component can be developed as a reliable marker for the determination of appropriate ASV doses in management of snakebite cases.

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