ORIGINAL ARTICLE

Nucleotidase as a Clinical Prognostic Marker in Snakebites: A Prospective Study

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Abstract

Background: Snakebite envenomation is a critical global health issue, causing substantial mortality and morbidity. Snake venom includes various enzymes, such as nucleotidase, phosphatases, etc. which impact physiological functions. However, research on the role of serum 5′-nucleotidase levels in assessing the severity and outcomes of snakebites is limited. This study aims to measure serum 5′-nucleotidase levels and explore their correlation with the severity of envenomation, to better understand its role in predicting patient prognosis.

Methods: This is a single-center, prospective observational analysis involving 82 snakebite patients. Serum 5′-nucleotidase levels were measured using enzyme-linked immunosorbent assay, and clinical severity was evaluated using the snakebite severity score (SSS). Statistical analyses were performed to determine the correlation between 5′-nucleotidase levels and SSS, as well as various complications.

Results: Among the 82 snakebite patients, 71.9% were male and 28.1% were female. Most bites (62.2%) occurred during the day, and 83% involved the lower limbs. Recovery was high, with 93.9% discharged, 3.7% deceased, and 2.4% lost to follow-up. A positive correlation was observed between 5′-nucleotidase levels and SSS at both 0 and 24 hours, with correlation coefficients of 0.55 and 0.61, respectively ($p < 0.001$).

Conclusion: Serum 5′-nucleotidase serves as an effective biomarker for assessing the severity of snakebite envenomation and predicting patient outcomes. Its strong correlation with clinical severity scores makes it a valuable tool for improving the prognostication and management of snakebite cases when used in conjunction with clinical assessments.

Keywords: Envenomation, Neurotoxin, Nucleotidase, Snakebite, Snake venom.

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Highlights

- The study identifies serum 5′-nucleotidase as a valuable biomarker for assessing the severity of snakebite envenomation.
- A significant positive correlation was found between 5′-nucleotidase levels and the snakebite severity score, with correlation coefficients of 0.55 and 0.61 at 0 and 24 hours, respectively.
- Higher levels of serum 5′-nucleotidase are associated with more severe envenomation, suggesting its potential use in predicting patient prognosis.
- Incorporating 5′-nucleotidase measurements into clinical practice could improve early intervention strategies, optimize treatment, and enhance patient management.
- When used alongside clinical severity scores, serum 5′-nucleotidase provides an objective biochemical parameter that strengthens the overall assessment of envenomation severity.

Introduction

Snakebite is a significant but often overlooked environmental hazard with high rates of morbidity and mortality. It is a serious medical emergency that has led to numerous hospital admissions across various parts of Asia.[1] In 2017, the World Health Organization recognized snakebite as a neglected tropical disease, highlighting its significance alongside other infectious diseases.[2] Consequently, the World Health Assembly passed a resolution in 2018 to address snakebite issues in the South Asian region.[3,4] In India, snakebite is a major public health problem, predominantly affecting rural populations, including agricultural workers and forest-dwelling communities.[4] Snake envenomation can lead to severe complications,

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often requiring hospitalization and, in some cases, resulting in death. Contributing factors to the high morbidity and mortality rates include delayed access to appropriate medical care, insufficient antivenom availability, and limited supportive treatments.[1] In India, it is estimated that around 1.2 million snakebite deaths have occurred over the past decade, averaging 58,000 per year.[5] This figure is based on hospital statistics, but many snakebite victims seek treatment from traditional healers rather than visiting hospitals.

Current treatment strategies for snakebite cases rely on clinical features, but managing complications across different physiological systems due to envenomation remains a topic of debate. Snake venoms contain various enzymes produced by specialized glands, which play a role in defense mechanisms against predators and in immobilizing or killing prey.

Of these, 5′-nucleotidase is widely distributed across snake venoms, indicating its crucial role in envenomation strategies. This enzyme is consistently present in all four medically significant snake species in India, which are responsible for most cases of morbidity and mortality in the Indian subcontinent.[6,7] Despite its importance, 5′-nucleotidase has not received significant attention in snakebite research. Currently, the primary treatment for snakebites is the intravenous administration of antivenom anti-snake venom (ASV), guided by clinical assessment and whole blood clotting time. However, venom components in serum are not yet considered or measured in the management of snakebites. This study aims to evaluate the role of serum 5′-nucleotidase, alongside clinical assessment and whole blood clotting time, to determine its utility in predicting patient outcomes following a snakebite.

Materials and Methods

Sample Source

This study was conducted in the Department of General Medicine at the Karnataka Institute of Medical Sciences, Hubballi. Patients were selected from those admitted to the hospital's inpatient department with a history of snakebite, meeting the inclusion criteria: being over 18 years old and providing informed consent, or under 18 years old with consent from a guardian. Patients were included if they had a whole blood clotting test result greater than 20 minutes, exhibited local reactions, such as swelling, cellulitis, gangrene, blebs, or bleeding at the bite site, and showed any signs of systemic envenomation. Clinical severity was assessed using a score based on characteristic clinical signs and symptoms of snakebite, and further routine tests were conducted as determined by clinical evaluation.

Enzyme-linked Immunosorbent Assay (ELISA)

Serum samples were collected from patients both before and 24 hours after the ASV to measure the levels of snake venom-specific 5′-nucleotidase enzyme using the ELISA method, in accordance with the manufacturer's instructions from Sincere Biotech. The entire procedure took approximately 1 hour and 30 minutes. Each well of a BD Falcon™ microplate was coated with 40 µL of soluble proteins from the patient's serum and incubated with a precoated plate containing specific 5′-nucleotidase antibodies. A secondary antibody, conjugated with horseradish peroxidase (HRP), was added to create a sandwich complex. After incubation and washing, the reaction was developed by adding 3,3′,5,5′-tetramethylbenzidine substrate to catalyze the HRP enzyme. The intensity of the blue color that developed was measured at 450 nm using an iMark microplate absorbance reader (BioRad). The concentration of 5′-nucleotidase in the samples was calculated by comparing the measurements to standard curves and was reported in mg/mL.

Results

In this study, a total of 82 snakebite patients were evaluated, including 59 males and 23 females. The patients were categorized into four age-groups: under 30 years, 31–45 years, 46–60 years, and over 60 years. Specifically, there were 17 patients under 30 years, 29 patients aged 31–45 years, 23 patients aged 46–60 years, and 13 patients over 60 years. Among the 82 patients, 51 reported their snakebites occurring during the day, while 31 experienced their bites at night. Most snakebites occurred on the lower limbs (68 cases) compared to the upper limbs (13 cases), and 1 patient had a snakebite on the head region.

Snakebite Severity Score (SSS)

The SSS was evaluated using a grading system from 0 to 4, based on clinical symptoms observed in six different categories: local wound, pulmonary, cardiovascular, gastrointestinal, hematological, and central nervous system manifestations.[8] In this study, the SSS was classified into three categories: minimal, moderate, and severe, corresponding to grades I, II, and III or IV, respectively. However, according to standard snakebite protocols, ASV is not recommended for patients with a severity score of grade 0, and so these cases were not included in the study.

The severity score was recorded at two time points: before and 24 hours of standard treatment as per the current guidelines. Among the 82 patients included in the study, 24 presented with a minimal severity score, 47 with a moderate severity score, and 11 with a severe severity score at the time of admission. However, the severity scores showed that 53 patients had minimal symptoms, 25 had moderate symptoms, and 4 had severe symptoms after 24 hours of standard treatment.

ASV Administration

All admitted patients were administered with different dosages of ASV depending upon the whole blood clotting time and severity score. In the present study, out of 82 patients, 10 patients received 10 vials of ASV, 49 patients received 20 vials of ASV, and 23 patients received 30 vials of ASV doses. Further, it was observed that 11 patients developed allergic reactions to ASV, ranging from minor rashes to severe anaphylaxis.

Snakebite Local Signs and Symptoms

In the present study, local signs and symptoms on bite site were recorded, which showed that pain symptoms in bite site was the predominantly present in most number of patients (41), followed by signs of bleeding from the bite site (39), and swelling in the bite site (36); however, 10 patients were not having any local signs/symptoms, further 9 patients were recorded with paresthesia, and around 7 patients developed blisters/blebs around the wound site, and 2 patients presented with discoloration signs around the bite site.

Correlation of Snake Venom-specific 5′-Nucleotidase Levels and Severity Score

In the present study, snake venom-specific 5′-nucleotidase was quantified by the ELISA method, which revealed that 5′-nucleotidase was in the range of 10–76 ng/mL before administration of ASV. However, the concentration of 5′-nucleotidase after 24 hours of ASV administration was estimated to be in the range of 8–78 ng/mL.

Further in the present study, to know the correlation between severity score and the level of venom-specific 5′-nucleotidase enzyme in blood of snakebite patients, the mean value of venom-specific 5′-nucleotidase levels were recorded with all three severity score categories before and after 24 hours of ASV administration as shown in Table 1 and Figure 1.

5′-nucleotidase Levels in Severe Envenomation Treatment

In addition to standard care, management strategies for snakebite patients vary considerably depending on the complications that develop. However, patients with a greater number of clinical symptoms often require multiple management strategies. In this study, the mean levels of venom-specific 5′-nucleotidase were measured at two time points: before the initiation of standard care (0 hours) and 24 hours after receiving standard care, in conjunction with various management strategies represented in Figure 2 and Tables 2 and 3.

For 11 patients requiring dialysis, the venom-specific 5′-nucleotidase level in blood samples was 25.5 ng/mL at 0 hours. After 24 hours of standard care, this level increased to 29.05 ng/mL. In 12 patients who underwent fasciotomy due to compartment syndrome, the 5′-nucleotidase level was 20.83 ng/mL before treatment and rose to 24.83 ng/mL after 24 hours of therapy.

Similarly, among 48 patients treated with magnesium sulfate ($MgSO_4$) dressings for cellulitis, the venom-specific 5′-nucleotidase levels were 18.01 ng/mL before treatment and 19.83 ng/mL after 24 hours of care. For 11 patients who required invasive mechanical ventilation, the 5′-nucleotidase level was 21.18 ng/mL before ventilation and increased to 22.82 ng/mL after 24 hours of support. Additionally, in 19 patients who received blood or blood product transfusions, the 5′-nucleotidase level was 22.55 ng/mL before transfusion and increased to 24.63 ng/mL after 24 hours.

In contrast, among 71 patients who received standard care without requiring dialysis, the 5′-nucleotidase levels were 14.62 ng/mL at 0 hours and 15.55 ng/mL after 24 hours. Similarly, in 70 patients without compartment syndrome, the levels were 15.26 ng/mL before treatment and 16.08 ng/mL after 24 hours. For 34 patients who did not exhibit cellulitis symptoms, the 5′-nucleotidase levels were 13.35 ng/mL before treatment and 13.87 ng/mL after 24 hours.

Among 71 patients who did not require invasive mechanical ventilation, the 5′-nucleotidase levels were 15.29 ng/mL at 0 hours and 16.51 ng/mL after 24 hours of standard care. Lastly,

Table 1: Snakebite severity score

Snakebite severity score	*Cases*
0 hour	
Minimal	24
Moderate	47
Severe	11
24 hours	
Minimal	53
Moderate	25
Severe	4

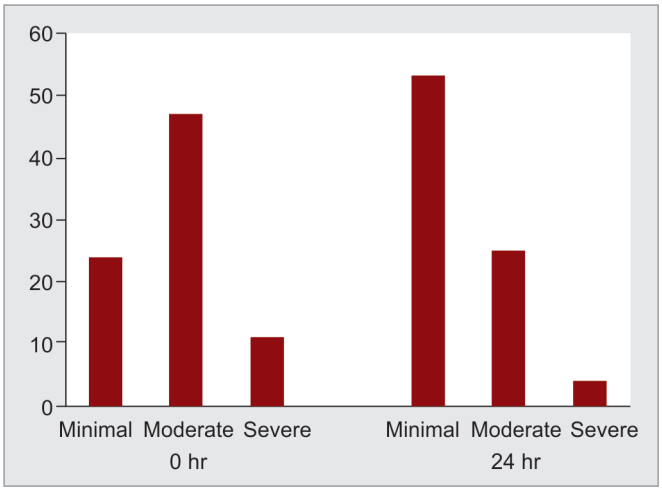
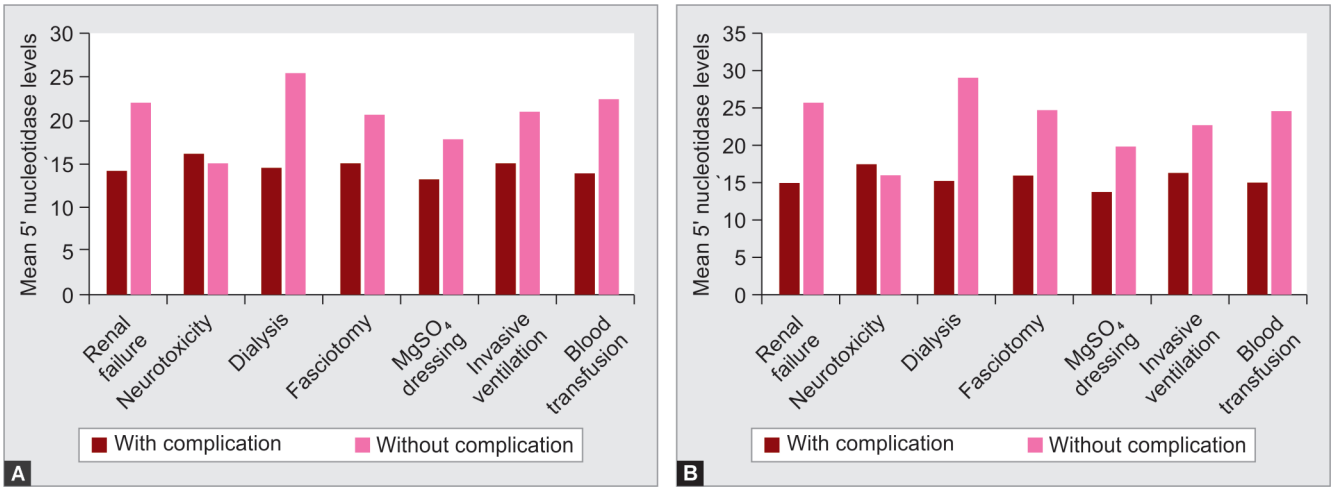


Fig. 1: Snakebite severity score categories at 0 and 24 hours of ASV administration



Figs 2A and B: Comparison of serum nucleotidase levels at 0 and 24 hours with and without complications of snakebite

Table 2: Serum 5′-nucleotidase levels at 0 hour with and without complications

Complications at 0 hour	*Mean 5′-nucleotidase levels*	
	With complication	*Without complication*
Renal failure	14.34	22.25
Neurotoxicity	16.27	15.18
Dialysis	14.62	25.5
Fasciotomy	15.26	20.83
$MgSO_4$ dressing	13.35	18.01
Invasive ventilation	15.29	21.18
Blood transfusion	14.13	22.55

Table 3: Serum 5′-nucleotidase levels at 24 hours with and without complications

Complications at 24 hours	*Mean 5′-nucleotidase levels*	
	With complication	*Without complication*
Renal failure	15.06	25.53
Neurotoxicity	17.58	16.29
Dialysis	15.55	29.05
Fasciotomy	16.08	24.83
$MgSO_4$ dressing	13.87	19.83
Invasive ventilation	16.51	22.82
Blood transfusion	15.17	24.63

in 63 patients who did not receive blood or blood product transfusions, the 5′-nucleotidase levels were 14.13 ng/mL before treatment and 15.17 ng/mL after 24 hours of care.

Discussion

Snakebite envenomation complications can vary significantly, ranging from a dry bite with no apparent clinical effects to life-threatening conditions. These severe effects can include coagulopathy, respiratory or heart-rate alterations, increased vascular permeability, and neurological symptoms, such as fasciculations, paresthesia, mental status depression, and weakness. Recent study showed that snake venom-specific phospholipase A2 in the blood of snakebite patients could be used as a biomarker for determining ASV dosage in snakebite patients.[9] Due to the lack of an appropriate tool to estimate real-time venom concentration in snakebite victims, current therapy primarily relies on the clinical condition of the patient, which directly correlates with the SSS.[10]

The SSS offers a more objective method for assessing the severity and progression of envenomation. However, it is important to note that the SSS was originally developed as a research tool to quantify snakebite manifestations. It does not provide an absolute value for detecting all envenomation complications, nor does it distinguish between complications arising from the snakebite itself and adverse effects associated with ASV, which are common in snakebite cases.[11,12]

In the present investigation, attempts were made to correlate the venom component 5′-nucleotidase with clinical complications and the SSS. The results revealed that the amount of venom-specific 5′-nucleotidase in the blood of snakebite victims was directly proportional to their recorded SSS. Additionally, the levels of 5′-nucleotidase measured in this study were also well correlated with the clinical complications experienced by the victims. Overall, analyzing the levels of venom-specific 5′-nucleotidase in the blood of snakebite patients provides a more objective tool for prognosticating outcomes in these cases.

However, while the findings of this study are promising, they underscore the need for further clinical research in the field of snakebite envenomation.

Conclusion

The study demonstrates that serum 5′-nucleotidase is a promising biomarker for evaluating the severity of snakebite envenomation and predicting patient outcomes. The positive correlation between 5′-nucleotidase levels and the SSS at both initial and follow-up intervals highlights its potential utility in clinical settings. By providing a more objective measurement that complements existing clinical assessments, the inclusion of serum 5′-nucleotidase levels in routine evaluations could enhance the accuracy of prognostication and inform treatment decisions. Further research in larger cohorts and diverse settings would help validate these findings and explore their broader applicability.

Clinical Significance

Snakebite envenomation is a significant public health concern, particularly in tropical and subtropical regions, where it leads to high mortality and morbidity rates. Currently, the assessment of envenomation severity relies heavily on clinical symptoms and scoring systems, such as the SSS. However, there is an urgent need for reliable biomarkers that can offer objective and early insights into patient prognosis, allowing for more accurate prediction of outcomes and timely interventions.

This study highlights the clinical significance of serum 5′-nucleotidase as a novel biomarker in snakebite envenomation. The positive correlation between serum 5′-nucleotidase levels and SSS indicates that this enzyme plays a critical role in reflecting the severity of envenomation. Its consistent correlation at both initial presentation and 24 hours after bite suggests that it can serve as a dynamic indicator for monitoring the progression of envenomation and guiding therapeutic decisions.

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Author Contributions

RSK, TH, and MA treated the patient. RSK consulted the patient. SB, MMK, GLV, and VS analyzed and interpreted the data and wrote the manuscript. All authors were involved in the analysis and interpretation of findings. All authors proofed the manuscript and contributed to important intellectual content. All authors contributed to writing and approved the final manuscript.

Data Availability Statement

The authors of this manuscript are willing to share the data supporting the results of this manuscript upon request.

Ethical Approval

The study was approved by Karnataka Institute of Medical Sciences, Hubballi Ethics Committee, Registration No. ECR/486/Inst/KA/2013/RR-16, approval date July 2021. The procedures in the study follow the guidelines laid down in Declaration of Helsinki 1964 and as revised later.

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References

1. World Health Organization. Regional action plan for prevention and control of snakebite envenoming in the South-East Asia 2022–2030. Geneva: World Health Organization; 2022.
2. Bawaskar HS. Snake bite poisoning: A neglected life-threatening occupational hazard. Indian J Crit Care Med 2014;18:123–124. DOI: 10.4103/0972-5229.128698.
3. Sachan D. The snake in the room: Snakebite's huge death toll demands a global response. BMJ 2018;5:361. DOI: 10.1136/bmj.k2449.
4. World Health Organization. Snakebite envenoming: Member states provide WHO with clear mandate for global action. Geneva: World Health Organization; 2018.
5. Mohapatra B, Warrell DA, Suraweera W, Bhatia P, Dhingra N, Jotkar RM, et al. Snakebite mortality in India: A nationally representative mortality survey. PLoS Negl Trop Dis 2011;5(4):e1018. DOI: 10.1371/journal.pntd.0001018.
6. World Health Organization. Study estimates more than one million Indians died from snakebite envenoming over past two decades. Geneva: World Health Organization; 2020.
7. Nallathambi M. A study of clinical and prognostic forecast in snake bite [dissertation]. Thanjavur, Tamil Nadu, India: Thanjavur Medical College; 2017.
8. Whitaker R, Whitaker S. Analysis of snakebite data from Pappinisseri Vishachikilsa Society, Kannur, Kerala (India). Calicut Med J 2006;4(2):e2. DOI: 10.4103/0028-3886.158155.
9. Kaulgud RS, Hasan T, Vanti GL, Veeresh S, Uppar AP, Kurjogi MM. Snake venom-specific phospholipase A2: A diagnostic marker for the management of snakebite cases. Indian J Crit Care Med 2022;26(12):1259–1266. DOI: 10.5005/jp-journals-10071-24362.
10. Dart R, Hurlbut K, Garcia R, Boren J. Snakebite severity score. Toxicon 1996;34(2):145. DOI: 10.1016/0041-0101(96)83658-0.
11. Alirol E, Sharma SK, Bawaskar HS, Kuch U, Chappuis F. Snake bite in South Asia: A review. PLoS Negl Trop Dis 2010;4(1):e603. DOI: 10.1371/journal.pntd.0000603.
12. de Silva HA, Ryan NM, de Silva HJ. Adverse reactions to snake antivenom, and their prevention and treatment. Br J Clin Pharmacol 2016;81(3):446–452. DOI: 10.1111/bcp.12739.