IntRoductIon

Acute kidney injury (AKI) remains a significant cause of morbidity and mortality among victims of snakebites due to Viperidae group and sea snakes. In India, venoms of Russell’s viper and Echis carinatus are the most common viper species that often cause acute tubular necrosis or cortical necrosis at times and rarely, a combination of both. AKI that occurs after snake envenomation is often reversible with supportive measures and injection anti-snake venom, whereas acute cortical necrosis if develops, it may lead to an incomplete recovery.

Alteration in glomerular filtration rate (GFR) is usually noticed indirectly by an increase in serum creatinine. Rising serum creatinine levels from basal level are considered as the gold standard for the diagnosis of impaired renal function in the current consensus definitions. A substantial rise in creatinine typically occurs after significant renal damage and can be even due to nonrenal factors. Any reduction in GFR leads to an increase in creatinine that requires three half-lives to reach a new equilibrium from which GFR can be estimated. Since the half-life of serum creatinine is about 4 h when GFR is normal, reliance on serum creatinine delays diagnosis in clinical practice by up to 72 h, particularly after modest injury.

As elevation of serum creatinine is delayed by day (s), it becomes a less reliable biomarker in the scenario of viper envenomation. Therefore, there is a need to find out better biomarkers to detect AKI early but also assisted to plan for appropriate intervention. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker to detect AKI in viper envenomation cases were not studied much. The present study was undertaken to find out plasma NGAL levels and assess its usefulness among the hospitalized Viperidae group of snakebite victims in predicting acute kidney injury.

Materials and Methods:
The plasma NGAL level was estimated within 6–8 h of all the 184 cases of viper bites along with other laboratory parameters.

Results:
It was elevated much before the elevation of serum creatinine levels, irrespective of the age, gender, and bite to hospital time. The sensitivity and specificity was 99.37 and 96.15, respectively. Elevated plasma NGAL levels in viper bite helped not only to detect AKI early but also assisted to plan for appropriate intervention.

Conclusion:
It is suggested to include estimation of plasma NGAL in the point of care testing, especially in emergency settings handling snakebite cases. However, more studies are recommended to find out its serial levels in snakebite cases following different kinds of snake envenomation with different clinical and laboratory manifestations in different age groups and gender belonging to different population so as to arrive at valid conclusions.

Keywords: Acute kidney injury, biomarker, diagnostic test, neutrophil gelatinase-associated lipocalin, neutrophil gelatinase-associated lipocalin, serum creatinine, viper bite

Abstract

Background: Snakebite envenomation is a major public health problem in the developing world, and the effects of viper envenomation on renal tissues leading to acute kidney injury (AKI) are well known. However, the usefulness of neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker to detect AKI in viper envenomation cases were not studied much. Aims and Objectives: The present study was undertaken to find out plasma NGAL levels and assess its usefulness among the hospitalized Viperidae group of snakebite victims in predicting acute kidney injury. Materials and Methods: The plasma NGAL level was estimated within 6–8 h of all the 184 cases of viper bites along with other laboratory parameters. Results: It was elevated much before the elevation of serum creatinine levels, irrespective of the age, gender, and bite to hospital time. The sensitivity and specificity was 99.37 and 96.15, respectively. Elevated plasma NGAL levels in viper bite helped not only to detect AKI early but also assisted to plan for appropriate intervention. Conclusion: It is suggested to include estimation of plasma NGAL in the point of care testing, especially in emergency settings handling snakebite cases. However, more studies are recommended to find out its serial levels in snakebite cases following different kinds of snake envenomation with different clinical and laboratory manifestations in different age groups and gender belonging to different population so as to arrive at valid conclusions.

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INTRODUCTION

Acute kidney injury (AKI) remains a significant cause of morbidity and mortality among victims of snakebites due to Viperidae group and sea snakes. In India, venoms of Russell’s viper and Echis carinatus are the most common viper species that often cause acute tubular necrosis or cortical necrosis at times and rarely, a combination of both. AKI that occurs after snake envenomation is often reversible with supportive measures and injection anti-snake venom, whereas acute cortical necrosis if develops, it may lead to an incomplete recovery.

Alteration in glomerular filtration rate (GFR) is usually noticed indirectly by an increase in serum creatinine. Rising serum creatinine levels from basal level are considered as the gold standard for the diagnosis of impaired renal function in the current consensus definitions. A substantial rise in creatinine typically occurs after significant renal damage and can be even due to nonrenal factors. Any reduction in GFR leads to an increase in creatinine that requires three half-lives to reach a new equilibrium from which GFR can be estimated. Since the half-life of serum creatinine is about 4 h when GFR is normal, reliance on serum creatinine delays diagnosis in clinical practice by up to 72 h, particularly after modest injury.

As elevation of serum creatinine is delayed by day (s), it becomes a less reliable biomarker in the scenario of viper envenomation. Therefore, there is a need to find out better biomarkers to recognize AKI at the earliest and institute appropriate measures, so as to reduce the morbidity and mortality associated with it.

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Neutrophil gelatinase-associated lipocalin (NGAL) was originally identified as a 25-kDa protein covalently bound to gelatinase of neutrophils. Processes induced by NGAL include bacteriostasis, anti-apoptotic effects, and an enhanced proliferation of renal tubules, which constitute possible pathways of NGAL-mediated kidney protection in acute injury. Although NGAL is expressed at very low levels in several human tissues, it is markedly elevated whenever renal tubular or cortical cells are injured either by drugs or toxins. Moreover, this biomarker plays a central role in the control and regulation of cell survival and proliferation. The expression of NGAL in proliferating and regenerating tubular epithelial cells indicates its role in the process of repair also.

**Aim**

The aims of the study was to find out the plasma NGAL levels among the hospitalized *Viperidae* group snakebite victims and assess its usefulness to detect AKI.

**Materials and Methods**

This prospective study was carried out in an emergency department (ED) of a tertiary care hospital from June 2012 to February 2018 with 184 established cases of viper envenomation presented within 6–8 h of bite, and their serum creatinine level was within 1.2 mg/dl on arrival. Snakebite victims whose serum creatinine level was >1.2 mg/dl, and those who had preexistent renal diseases, long-standing diabetes or hypertension, overt congestive heart failure (New York Heart Association III–IV), hemodynamic instability of any cause, sepsis or systemic infectious diseases, exposure to nephrotoxic drugs/chemicals, or other biological toxins and injuries were excluded from the study. Informed written consent was obtained from every participant before the enrollment. The study was performed in accordance with the Declaration of Helsinki and after the approval of the Institutional Ethics Committee.

Cases of viper bites were assessed clinically and then, subjected to laboratory evaluation such as complete blood count including clotting time, blood chemistry including plasma NGAL and serum creatinine, and urine for sediments and protein at the time of their arrival to ED. Their estimated GFR was calculated. Plasma NGAL was estimated using the standardized Triage® NGAL tests (Biosite Incorporated, San Diego, CA, USA). Serum creatinine was determined by Jaffe’s method. All the patients included in this study were monitored for hourly urine output and serial serum creatinine levels at 0, 12, 24, and 48 h and again at the time of discharge from the hospital. All these cases received anti-snake venom and supportive measures. During the hospital course, these patients did not receive any nephrotoxic agents. Throughout the study, good laboratory and clinical practice were adhered too. The data were analyzed statistically.

**Results**

There were 131 (71.1%) male and 53 (28.8%) female, and their age ranged from 16 to 80 with a mean and median of 38.12 and 54 years, respectively. Mean body weight of males and females included in the study was 65 and 55 kg, respectively. On arrival, all these cases had local symptoms and signs of envenomation without any systemic manifestations, and their hematology and blood chemistry were within acceptable limits, but for prolonged clotting time. Their plasma NGAL and serum creatinine levels on arrival ranged from 210 to 615 ngm/dl and 0.8 to 1.1 mgs/dl with a mean ± standard deviation (SD) of 278.3 ± 62.5 ngm/dl and 0.9 ± 0.3 mgs/dl, respectively. The plasma NGAL level was elevated significantly (*P* < 0.001) when compared with healthy control in snakebite victims even at the time of arrival, though their serum creatinine and estimated GFR were well within normal levels at that time. Serial estimation of serum creatinine levels varied from 1.1 to 2.1, with a mean ± SD of 0.40 mg/dl. During the hospital stay, their urine output was satisfactory and urinalysis was not contributory.

Based on the 24 h postadmission serum creatinine levels, the cases were considered to have developed nonoliguric AKI, and hence, an attempt was made to compare these levels with initial NGAL levels. As NGAL was elevated in all these cases, a receiver operator characteristic (ROC) curve was attempted and the details are furnished in Table 1. The ROC for plasma NGAL yielded a sensitivity of 99.37% and specificity of 96.15% for a cutoff value of 245 ngm/dl. As the positive predictive value to detect AKI was 99.37% among the ethnic study population, it is likely that viper bite cases with plasma NGAL level of 245 ngm/dl or more were considered to be suffering from AKI requiring appropriate care and intervention including hemodialysis. During their hospital stay, none developed any other complications including death.

**Discussion**

Snakebite-induced AKI is an important cause of mortality in rural areas. Estimation of NGAL in the detection of AKI and the mechanisms involved for the elevation of it has been highlighted earlier in various clinical scenario. Our previous pilot study has also revealed that plasma NGAL was significantly (*P* < 0.001) elevated in viper bite victims when compared with nonvenomous snakebite cases and much before the elevation of serum creatinine. As elevation of NGAL

| NGAL values (ngm/dl) | Serum creatinine (mgms %) |
|-----------------------|--------------------------|
| >245                  | 157                      |
| <245                  | 1                        |
| Total                 | 158                      |

Sensitivity=a/(a+c)=99.37%, Specificity=d/(b+d)=96.15%, Positive predictive value=a/(a+b)=99.37%, Negative predictive value=d/(c+d)=96.15%, Accuracy=(a+d)/(a+b+c+d)=98.91%. NGAL: Neutrophil gelatinase-associated lipocalin.
was much before the elevation of serum creatinine with high sensitivity, plasma NGAL may be considered as a useful biomarker to detect AKI at the bedside among Viperidae group of snakebite victims. Preclinical transcriptome profiling studies identified NGAL as one of the most upregulated genes in the kidney very early after acute injury based on animal models. Plasma NGAL rises after the renal insult by the venom even before renal cell necrosis develops. This indicates that viper venom is toxic to renal cells.

In our study, the positive and negative predictive values were 99.37% and 96.15%, respectively. Hence, we strongly believe that elevated plasma NGAL is an early marker of AKI following viper envenomation and aids to diagnose AKI 48 h prior to the diagnosis based on RIFLE criteria. During the study, serum creatinine did not accurately reflect renal insult by viper venom until a steady state was reached by 24 or 48 h in different cases. At this juncture, one has to remember that the renal cellular response to viper venom may vary in different individuals because of the genomic nature and the chemokines involved in the production and release of NGAL vary from one another. The other contributory factors for cellular injury related to snake are the habits and age of the snake, amount of venom injected, and the constituents of venom in addition to season, time of bite, bite to hospital time, and time of investigation.

Thamarai and Sivakumar correlated the plasma NGAL levels of their viper bite cases with RIFLE criteria and observed higher levels of plasma NGAL (mean 517 ngm/dl) in patients who fell into the category of “failure” (RIFLE), whereas patients who fell into the category of injury had a mean plasma NGAL of 298.47 ngm/dl. In our study, we did not observe any correlation between the plasma NGAL and serum creatinine at the time of admission. In our study, the area for plasma NGAL under the ROC (AUC-ROC) was 0.96 (95% confidence interval: 0.93–0.97), thus suggesting that plasma NGAL is a better predictor over serum creatinine, in diagnosing AKI.

Conclusion
The present study demonstrated an elevation of plasma NGAL a biomarker even at 6–8 h after viper bite and its usefulness to detect AKI much before serum creatinine and for an appropriate intervention. In view of that it suggested that estimation of plasma NGAL may be included in the point of care testing, especially in emergency settings handling snakebite cases.

The limitations of the study are nonestimation of plasma NGAL serially due to technical constraints and cases belonged to two species of Viperidae group from a single center. The strengths of the study are confirmation of viper bite by identifying the snakes and the adoption of good laboratory and clinical practice. Further studies are suggested to estimate serial levels of NGAL in different kinds of snake envenomation with different clinical and laboratory manifestations in different age groups and gender belonging to different population so as to arrive at valid conclusions.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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